ANTHOCYANINS AND OTHER FLAVONOIDS IN *ANTHURIUM ANDRAEANUM* LINDEN EX ANDRÉ

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We certify that we have read this dissertation and that in our opinion it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Horticulture.

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Dedicated to my husband, Earl, and children, Tricia, Randal, and Daniel.

ABSTRACT

The major anthocyanin pigments of Anthurium andraeanum Linden ex André were identified by thin layer chromatography, spectroscopy and gas liquid chromatography as cyanidin 3-rhamnosylglucoside and pelargonidin 3-rhamnosylglucoside.

A noncyanic flavonoid was isolated by two-dimensional paper chromatography of a methanolic extract of the spathes. Using chromatography and ultraviolet spectroscopic shifts with selected reagents, the compound was characterized as acacetin 7-glycoside. No flavonol, aurone or chalcone was detected.

Quantitative and qualitative measurements were taken of the anthocyanins and the flavone in anthurium clones of the breeding program at the University of Hawaii. The major color classifications were related to specific anthocyanins and their concentrations.

Quantitative and qualitative measurements of the anthocyanins and the flavone were taken of the parent plants and progenies of three crosses. Segregations by color of the offspring of these crosses were analyzed and a scheme of inheritance was proposed. The scheme consists of a system of monogenic control of each anthocyanin, an incomplete dominance form of intra-allelic interaction involving dosage effects on the concentration of each pigment, and recessive epistasis. Results of preliminary tests of the viability of the scheme of inheritance generally supported the scheme.

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I. INTRODUCTION

Anthuriums are the most important cut flower crop in Hawaii today. In 1979 the wholesale value of anthurium flowers sold was over 5.6 million dollars (Garrett and Martin, 1980).

The major commercial cultivar is 'Ozaki' which has a light red spathe and light purple spadix. It has good yield and excellent commercially desirable qualities of color and spathe shape. When the plants are mature, the inflorescences have spathes which are large and command premium prices. Sucker production is good so that availability of stock for expanding acreage is adequate. This cultivar is not without its problems. The spadix tends to become upright with increasing maturity. There is a tendency toward fading and burning of the spathes and the vase life is comparatively poor. 'Ozaki' is susceptible to anthracnose (Aragaki et al., 1968).

Other red cultivars of commercial importance today include 'Kozohara' and 'Kaumana'. They both have good yield and good vase life. 'Kozohara' has good shape and is a vigorous grower, producing large spathes at plant maturity. 'Kaumana' produces small to medium flowers. Both of these red cultivars are susceptible to anthracnose and have shades of red which are not as commercially popular as that of 'Ozaki'.

The cultivar other than red which has been of major importance in the industry is 'Nitta' which has a bright orange spathe and yellow spadix. It is a vigorous grower, produces suckers readily, and the flower has good vase life. It has average yield and moderate resistance

to anthracnose. Internodes are relatively long, which is an undesirable trait in commercial production.

There have been a number of other cultivars of red, orange and other colors available to the industry. These include several University of Hawaii cultivars; however, they have not been established as major cultivars in the industry.

The need for improved cultivars in the areas of anthracnose resistance, high yields and new colors continues. In fact, top priority has been given to breeding for these qualities in the anthurium industry analysis prepared in 1980 (Kefford and Harada, 1980).

A. andraeanum, Linden ex André is an outcrossing species with a fairly long generation time, taking 1½ to 2 years from germination to flowering. The widely cultivated anthuriums are thought to be of hybrid origin (Kaneko and Kamemoto, 1978). Thus, genetic analysis and manipulations are extremely complex. Elucidation of the chemical factors involved in spathe color is a fundamental step which can facilitate further breeding endeavors.

An intense anthurium breeding program has been conducted at the University of Hawaii (Kamemoto and Nakasone, 1955, 1963; Kamemoto et al., 1968; Sheffer, 1974; Sheffer and Kamemoto, 1967; Sheffer and Kamemoto, 1977). The inheritance of spathe color has been studied, scoring the spathe color by visual means. At one time it was suggested that spathe colors were a trait governed by a single multiple allelic gene, and a scheme of spathe color inheritance was summarized in a report by Kamemoto et al. (1968). However, in a report on the inheritance of purple and white spathe colors of interspecific crosses Sheffer and Kamemoto (1977) suggested that more than one pair of genes

are involved in spathe color. A scheme of inheritance incorporating this idea has not been reported. At this time it appears that a biochemical approach to this problem might yield fruitful results. The objectives of this study were to qualitatively and quantitatively describe the chemical factors involved in spathe color and to propose a scheme of inheritance of spathe color which may be of help in further breeding work.

II. LITERATURE REVIEW

2.1 The flavonoids

There has been a great deal of work done in the area of flavonoid chemistry and biochemistry with excellent reviews on the isolation and identification of these compounds (Harborne, 1958a, 1958b; Geissman, 1962; Harborne, 1967; Mabry et al., 1970; Ribereau-Gayon, 1972; Harborne et al., 1975; Goodwin, 1976). This review summarizes those factors which are most germane to the work presented in this discussion.

The flavonoids are a large group of phenolic compounds, members of which are frequently responsible for yellow, red, orange, scarlet, mauve and blue color in plants. The flavonoids which are involved in flower color are the glycosides of anthocyanidins, flavonols, flavones, chalcones and aurones.

The anthocyanins are water soluble pigments located in vacuolar sap and are responsible for cyanic colors. They occur in plants as glycosides of anthocyanidins which are based on the flavylium cation (Figure 1).

On spectral analyses anthocyanidins have two main absorption maxima, one in the visible region between 465 and 550 nm and the other in the UV at about 270 nm. B-ring hydroxylation results in a bluing effect, the greater the degree of hydroxylation, the larger the bluing effect so that pelargonidin (4'-OH) is orange, cyanidin (3',4'-OH) is magenta and delphinidin (3',4',5'-OH) is purple).

In nature the anthocyanidins are always in glycosylated form which is the more stable structural form. Glycosylation is most common

at the 3-OH position but the 5- and 7- positions are also frequently glycosylated. The sugar residues may be monosides (most commonly glucose, galactose, rhamnose and arabinose), biosides (rhamnosylglucoside, xylosylglucoside, xylosylgalactoside, sophoroside and gentiobioside) or triosides with linear (gentiotrioside) and branched structures.

Glycosylation causes a hypsochromic shift with a reddening effect. The nature of the sugar does not influence this shift; however, different positions of glycosylation influence other characteristics of the spectrum such as the magnitude of a minor deflection in the 400-460 nm region or the magnitude of the short wave minimum. Under UV irradiation the 3-,5-diglucosides are intensely fluorescent and the 3-glucosides have a duller color.

Acid hydrolysis cleaves the sugar residues one at a time so that serial chromatographic analysis of the anthocyanin product during hydrolysis readily demonstrates the number of sugar residues involved. As each sugar moiety is released, the R_f of the anthocyanin changes until the aglycone is obtained, its R_f also differing from that of the monoglycoside in one or another solvent system.

Identification of anthocyanins is most frequently obtained by chromatographic and spectrophotometric criteria. Sometimes NMR (nuclear magnetic resonance) and MS (mass spectroscopy) are also used.

The color of the spot on the chromatogram gives the initial clue as to the anthocyanidin involved.

The R_f's of anthocyanins are taken before and after acid hydrolysis in a number of solvent systems. Spectral analysis includes recording

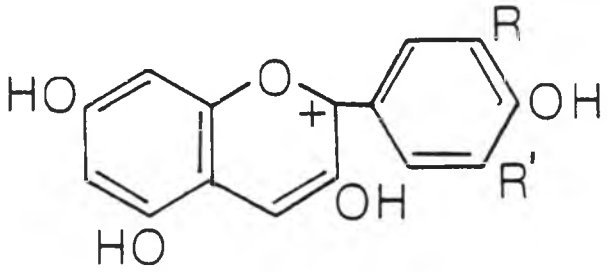
the entire spectrum in both the ultraviolet and visible wavelengths and observing for a bathochromic shift after the addition of a few drops of aluminum chloride solution. Free ortho-dihydroxyl groupings result in a bathochromic shift of some magnitude (18-52 nm) (Harborne, 1967). R_f values and absorption maxima are usually sufficient to determine the anthocyanidin.

The most common glycosides are the 3- and 3-,5-diglycosides. They can be distinguished by observing the difference in optical density in the 400-460 nm region. The 3-glycosides and free anthocyanidins usually have twice the absorption value as the 3-,5-diglycosides in that region. Another difference is in the intensity of the short-wave maximum. The 3-glycosides have a dull appearance under ultraviolet irradiation whereas the 3-,5- and 5-glycosides give some degree of fluorescence (Harborne, 1967).

The sugars may be identified by paper chromatography or by gas chromatography. In preparing the sugars for gas chromatography they are rendered volatile by silylation with tri-methyl silane. Comparison of retention times with similarly treated standards provides evidence for the identity of the sugar (Sweely, 1963).

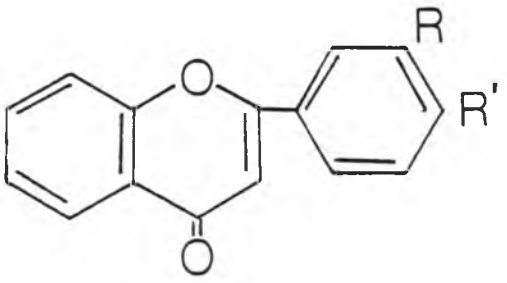
Flavones and flavonols are widely distributed yellow pigments which give substance to white flowers. Sometimes they are responsible for yellow flower color. They are most frequently active as co-pigments with anthocyanins. Structurally, the central C_3 ring in flavones and flavonols is more highly oxidized than anthocyanidin (Figure 1).

Both flavones and flavonols have structural variations corresponding to those found in anthocyanidins. However, variations are more



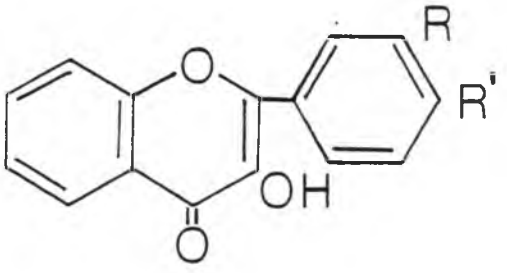
Common anthocyanidins:

Pelargonidin	R=R'=H
Cyanidin	R=OH, R'=H
Delphinidin	R=R'=OH



Common flavones:

Apigenin	R=H, R'=OH
Luteolin	R=R'=OH



Common flavonols:

Kaempferol	R=H, R'=OH
Quercetin	R=R'=OH

Figure 1. Structures of common anthocyanidins, flavones, and flavonols.

considerable. Many flavones which are partly or fully methylated as methyl ethers have been described as have been many C-glycosyl forms. The absorption maxima of flavones are in the range 330-350 and 250-270 nm. The flavonols have slightly higher maxima, in the range 350-390 and 250-270 nm.

Chalcones and aurones are bright yellow pigments which are more restricted in distribution. They have maxima in the range 365-430 nm and subsidiary maxima about 240-270 nm (ca. 30%). They are yellow pigments which are occasionally responsible for bright yellow colors in petals. They are easily detected by fuming the flower with ammonia and observing an orange or red transformation. The parent structures of these two pigments are presented in Figure 2.

Flavanones, flavanonols and isoflavones are flavonoids which are not known to contribute to flower color in a primary way. Absorption maxima for flavanones and flavanonols are in the range 275-290 and ca. 225 nm with a subsidiary maximum in the range 310-330 nm (ca. 30%). Absorption maximum for isoflavones falls in the range 255-265 nm with a subsidiary maximum in the range 310-330 nm. Parent structures are shown in Figure 2.

The noncyanic flavonoids are generally more stable than anthocyanins and are in that sense easier to work with. Identification of these flavonoids can be done following procedures similar to those for anthocyanins.

The color of the spot on the chromatogram under ultraviolet illumination before and after fuming with ammonia can frequently provide an adequate basis for establishing the general class of

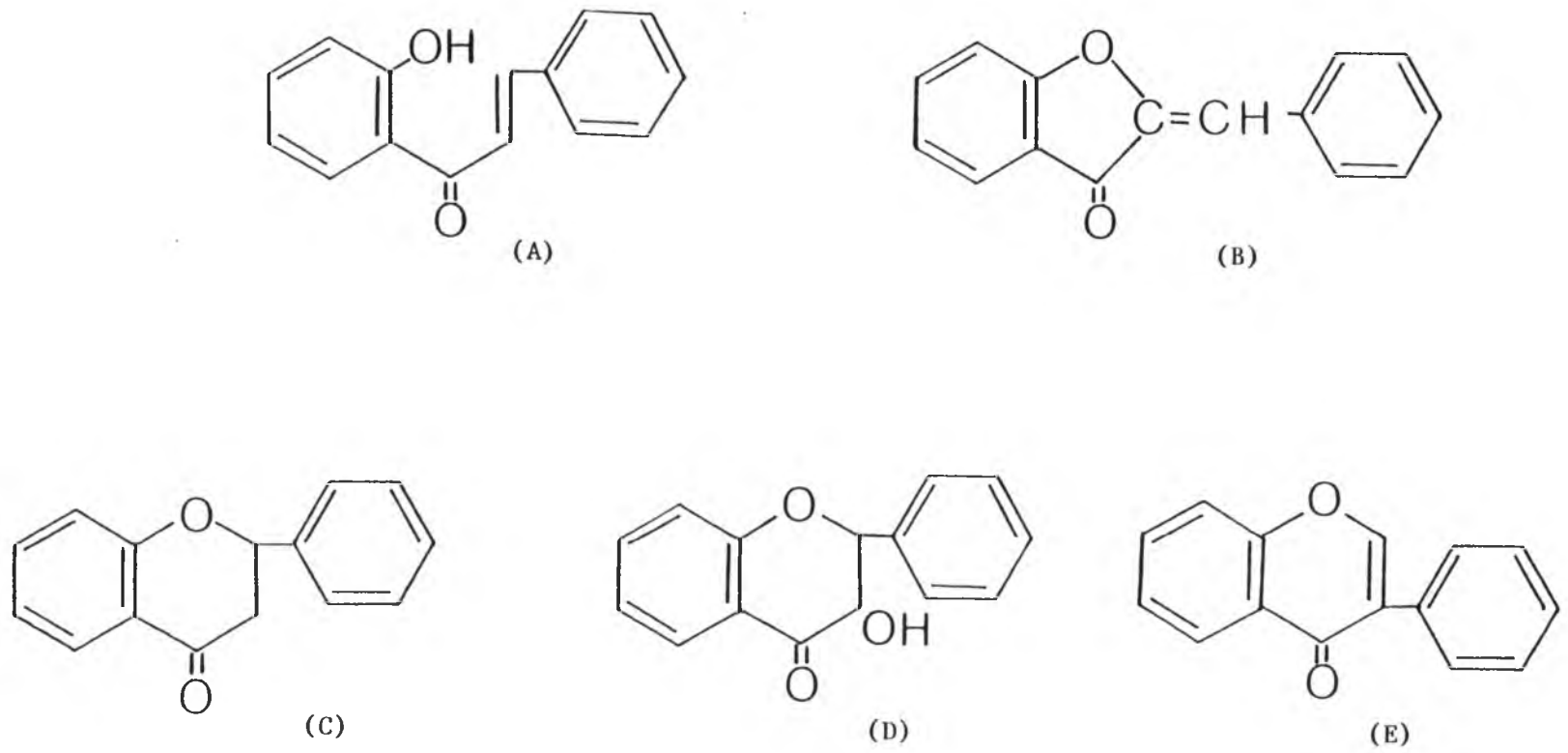


Figure 2. Structures of (A) chalcone, (B) aurone, (C) flavanone, (D) flavanonol and (E) isoflavone.

flavonoid. Viewing color changes after spraying with a variety of reagents can give further information about the class of compound.

The R_f and results of co-chromatography in a number of different solvent systems before and after acid hydrolysis are important. Spectrophotometric analyses are more extensive than with the anthocyanins and are of central importance to the characterization of these flavonoids.

Flavones and flavonols have two absorption maxima; the one at the longer wavelength is referred to as Band I and the one at the shorter wavelength is referred to as Band II. A-ring substitution by hydroxyl or methoxyl groups results in bathochromic shifts of Band II with an increased intensity of absorption. B-ring substitution by hydroxyl and methoxyl groups results in bathochromic shifts of Band I and an increased intensity of absorption (Jurd, 1962). Band I is thus associated with absorption due to the B-ring cinnamoyl system and Band II with the A-ring benzoyl system (Mabry et al., 1970).

Flavones and falvonols with a single 4'-substituent have a single well-defined Band II peak. However, when there are hydroxyl or methoxyl groups in both the 3'- and 4'- positions, Band II shows two peaks or one peak and an obvious inflection. With 3- substituents in the B-ring, Band II has a single peak (Jurd, 1962).

Several reagents can be employed to produce shifts in spectral maxima indicating the location of functional groups.

Bathochromic shifts with the addition of a few drops of $AlCl_3$ solution indicates free 5- and 3-hydroxyl groups as well as free ortho-dihydroxyl group in the B-ring (Giannasi, 1975). In flavones with a

free 5-OH, a complex forms with the 5-OH and 4-keto group resulting in a moderate bathochromic shift (20-45 nm) and a splitting of Band I into two peaks or a peak and an inflection. With flavonols having a free 3-OH, the bathochromic shift is much greater (60 nm) and there is no concomitant splitting of the peak. If 3'-4'-OH substituents are present there is a larger shift. In this case the addition of HCl in trace amounts breaks the acid-labile ortho-dihydroxyl complex and leaves the acid-stable 3-or 5-hydroxy, 4-keto complex so that a slight hypsochromic shift occurs (Giannasi, 1975).

Sodium acetate ionizes hydroxyls at positions 3, 7 and 4' of the flavone nucleus but does not affect hydroxyls at any other position. A small bathochromic shift (8-20 nm) is observed in Band II when a free 7-hydroxyl is present. None is observed if there is glucose or methyl substitution on the hydroxyl.

This reagent specifically tests for a free 7-hydroxyl. With free 3- and 4'-hydroxyls the compound decomposes with a collapse of the Band I peak.

Boric acid added to sodium acetate solution to the point of saturation tests for a free ortho-dihydroxyl group, usually in the 3'-, 4'- B-ring positions. It confirms results obtained from using $AlCl_3$ solution. In flavones and flavonols with the ortho-dihydroxyl group an 18-25 nm shift in Band I is observed.

Sodium methoxide is a powerful base which ionizes all free hydroxyls and is therefore a nonspecific reagent. However, a free 4'-hydroxyl is indicated by a large bathochromic shift (40-65 nm) without a decrease in intensity of the peak (Mabry et al., 1970). With free 3'-, 4'- dihydroxyl

group the Band I maximum collapses. Flavones with 4'-substitution by a methyl or sugar group show a decrease in intensity or collapse of Band I. Flavones with this *para*-hydroxyl substitution also do not fluoresce when fumed with ammonia (Giannasi, 1975).

In lieu of the above identification procedures, NMR, MS and HPLC (High Performance Liquid Chromatography) are increasingly being used.

2.2 *Factors which influence color*

Under ordinary illumination color may be influenced by such factors as the amount and kinds of anthocyanins, pH, metal chelation, co-pigmentation, and the presence of background pigments such as chlorophyll and carotenoids.

The range of anthocyanin concentration in petals is 0.01-15% of the dry weight (Harborne, 1976). Geissman and Mehlquist (1947) clearly demonstrated the effect of differing concentrations of anthocyanin on the shade of the observed color. They found that in the red genotypes designated salmon-red, standard-red and deep-red, all were pigmented with pelargonidin but the pigment concentration was in the ratio 1:2:4 for the salmon-red to standard-red to deep-red. The kind of anthocyanin is primarily determined by the oxidation of the B-ring, and since the absorption maxima increase with increased oxidation, the color perceived obviously changes toward blue. Methylation results in a reddening effect.

The influence of pH on observed color has long been established. Scott-Moncrief (1936) established that the red types in *Primula sinensis* have a low pH (5.4) and blue types a high pH (6.0). Beale *et al.* (1939) found in *Lathyrus odoratus* that all types containing the recessive factor

dull (d) were markedly bluer than those having the dominant factor. All types carried the same anthocyanins and the same co-pigments. The only major difference was a difference in pH of about 0.6 pH units. Asen et al. (1971), taking spectra of intact cells and measuring vacuolar pH, found that an increase in pH is the major factor associated with bluing of roses during aging. They found an increase of 0.7 pH units during the aging and bluing of roses beyond the initial pH of 3.7-4.15 of freshly harvested tissues. Similar correlations were found in geranium and larkspur (Stewart et al, 1975).

Aluminum, molybdenum or iron complexes of anthocyanidins having a free ortho-dihydroxyl system have been isolated from blue-flowered plants such as the blue cornflower (Asen and Jurd, 1967). Hydrangea is vivid red, but if given aluminum sulfate in soil having a pH of 5 or less, the blossoms become a deep gentian blue.

The phenomenon of co-pigmentation is one in which a pigment having little visible color associates with anthocyanin to produce a bathochromic shift and a bluing effect. DeLoose (1970) demonstrated that the difference between scarlet and orange versus bluish-red and magenta blossoms of Rhododendron simsii is in the amount of the flavonol pigment, the bluish-red and magenta having a higher content than the scarlet red.

Asen et al. (1972) demonstrated co-pigmentation effects by combining anthocyanin with flavones in vitro, obtaining a bathochromic shift until the absorption spectrum matched that of the intact cell.

With increasing pH anthocyanidin is converted via an unstable anhydrobase which has a bluer color to a colorless carbinol base (Jurd and Asen, 1966). Cell sap pH is most frequently 4.5-5.5 at which range

the anthocyanins would presumably undergo change to the colorless carbinol base and thus be virtually colorless. Asen et al. (1972) investigated co-pigmentation effects in relation to cell pH. They used the flavonol quercetin (which is essentially colorless) as the co-pigment and combined it with six common anthocyanins. They found two effects of co-pigmentation: (1) a bathochromic shift, and (2) a large increase in absorbance and concluded that the two co-pigmentation effects were a result of the stabilizing effect on the anhydrobase. Unlike metal complexing, the ortho-dihydroxyl system is not necessary for co-pigmentation.

The quantitative co-pigmentation effect, i.e., the degree of change in absorption maximum change and change in optical density is a function of anthocyanin concentration and ratio of co-pigment to anthocyanin; the greater the ratio, the greater the increment in optical density and absorbance maximum.

At pH 3.16 an increase in concentration of anthocyanin from 10^{-4} to 10^{-2} M resulted in an increase of optical density by 300 times, thus anthocyanins may undergo self-association in aqueous solutions.

The effect of anthocyanin on a carotenoid background is often brown as found in Cheiranthus cheiri (wallflower), Primula polyanthus and in the rose 'Cafe' (Harborne, 1976).

2.3 Inheritance of flower color

A number of reviews on the inheritance of flower color and/or flavonoids are available (Alston, 1964; Harborne, 1962, 1967). Although there is a basic pattern of flower color genetics, slightly different systems of inheritance are found among the different plants. Genes which

control flower color include those which modify anthocyanin structure, those which influence the availability of metal ions, those which control the pattern of distribution, and those which influence the quantity of individual and/or multiple pigments. Single and independent genes, serial gene systems, and/or allelic series may be involved (Harborne, 1967). In Zea mays, some 25 genes affecting flavonoid synthesis involving concentrations, tissue specificities and patterns within tissues have been identified (Styles and Ceska, 1977). Several of these genes appear regulatory in nature (Styles and Ceska, 1977; Fincham and Sastry, 1974).

Of particular interest here are genes controlling B ring hydroxylation in anthocyanins. Genes which control 3'-hydroxylation include the gene M in Antirrhinum majus (Geissman et al., 1954), I in Streptocarpus (Lawrence and Sturgess, 1957), R in Dianthus caryophyllus (Geissman and Mehlquist, 1947) and Sm in Lathyrus odoratus (Beale et al., 1939). It has generally been found that the more hydroxylated forms are dominant to the less hydroxylated and that oxidation of the B ring is not always complete, that is, genotypes with a gene for trihydroxylated anthocyanins, for example, also contain some di- and mono-hydroxylated forms of anthocyanin as well (Harborne, 1962). Contrary to this is the situation in Papaver rhoeas in which the dominant allele F governs pelargonidin production, cyanidin which is the more hydroxylated form being produced in the presence of the recessive allele f and the dominant allele E (Scott-Moncrief, 1936). The hydroxylation gene a^d in flax (Linum usitatissimum) is unusual in its action. When dominant both delphinidin and cyanidin are produced. When recessive, both pelargonidin

and cyanidin are formed. Dubois and Harborne (1975) suggested that the gene controls the enzyme which oxidates cyanidin to delphinidin and that it is epistatic to another gene concerned with pelargonidin synthesis so that pelargonidin is produced only when the gene $\underline{a}^{d}$ is in the recessive state.

A number of genetic studies of quantitative variation in anthocyanins have been done. Harborne and Sherratt (1961) found monogenic control over quantitative variation of anthocyanins in Primula sinensis where the mutant phenotype (orange) contained three times as much pelargonidin glucoside as the non-mutant (coral) form. Such monogenic control also appears in mauve forms of Solanum iopetalum where there is a four-fold difference in anthocyanin concentration (Harborne, 1976). Endo (1962) found a concentration difference of nine to ten times in Torenia fournieri to be controlled by two complementary genes. It has been a general observation that the effect of genes involved in anthocyanin production is factorial.

It has also been generally noted that heterozygotes have a lower level of pigments than homozygous dominants and that the presence of dominant pigment genes stimulates general pigment production (Harborne, 1962). There have been some cases where there is an obvious competition effect by the several pigments for a common C_{15} precursor. This occurs for example, in Antirrhinum (Jorgensen and Geissman, 1955) where the concentration of cyanidin 3-rutinoside varies inversely with the concentration of aurone. In other cases the concentration of individual pigments can vary without corresponding differences in the concentrations of other pigments. This is the case in Primula sinensis

(Harborne and Sherratt, 1961). In the former case the action of the gene results in increased production of a common precursor, and in the latter, the effect is directly on the enzyme controlling anthocyanin synthesis (Lawrence and Sturgess, 1957; Harborne and Sherratt, 1961).

In Lathyrus odoratus (Beale et al., 1939) seven factors associated with differences in the concentration of various flavonoids have been described. Some of these factors are associated with increasing the concentration of pigment, some with decreasing concentrations, and some show various interactions. A dominant factor such as D in Primula sinensis (Scott-Moncrief, 1936) may suppress anthocyanin synthesis resulting in the white phenotype. In other cases, recessive factors may result in white. Some whites have traces of pigment inducible by environmental conditions which suggests the action of an inhibitor rather than the action of null alleles (Harborne, 1967).

In maize, the intensifier gene in causes increases in anthocyanin concentration. Gene dosage effects have been demonstrated most clearly in maize. In the triple homozygous state of the endosperm there is a five-fold increase in pelargonidin-containing aleurone and an eight-fold increase in cyanidin-containing aleurone tissue (Reddy and Peterson, 1978).

2.4 Environmental and physiologic factors affecting biosynthesis

Biosynthesis of flavonoids. A basic scheme of flavonoid biosynthesis is fairly well established (Wong, 1976; Grisebach and Hahlbrock, 1974; Hahlbrock and Grisebach, 1975). There are essentially three stages in the biosynthetic pathway: (1) formation of the C_{15} skeleton which involves the shikimic acid and malonate pathways,

(2) formation of the individual classes of flavonoids which involves variation of the central C_3 moiety, and (3) elaboration of each compound within the class. Hydroxylation of ring A is generally believed to occur in the second stage, i.e., before the formation of chalcone, and hydroxylation of the 4' carbon in the first stage some time before formation of the C_6C_3 unit. Hydroxylation of 3' and 5' carbons is complex and seems to occur at different stages, including the third stage. Methylation, glycosylation and alkylation apparently occur late in the biosynthetic scheme also.

Factors affecting anthocyanin production. A number of environmental and physiologic factors affect anthocyanin biosynthesis and therefore concentration. These include light, temperature, stress, tissue injury and mineral deficiency.

Light is a stimulant for a number of enzymes in the biosynthetic pathway (Hahlbrock et al., 1971; Hahlbrock et al., 1976; Ebel and Hahlbrock, 1977). It is a general observation that light enhances the formation of anthocyanins. The most striking example of this is in the skin of normally red apple varieties in which anthocyanin development is completely inhibited in the absence of light (Siegelman, 1964).

Anthocyanin production is generally enhanced by lower temperatures but there are instances where higher temperatures have enhanced anthocyanin biosynthesis. It has therefore been suggested that the optimal temperature for metabolism coincides with the optimal temperature for anthocyanin biosynthesis in different plants (Blank, 1958). Tissue damage, tumors and phosphorus deficiency are also known to stimulate anthocyanin synthesis.

2.5 Anthocyanins in anthurium

Using paper chromatographic methods, Forsyth and Simmonds (1954) reported the presence of three cyanidin glycosides and one pelargonidin glycoside in Anthurium andraeanum. Lowry (1972) reported the presence of both pelargonidin and cyanidin 3-rutinoside in the spathes of all the cultivars of A. andraeanum which he examined. Pelargonidin 3-rutinoside has been reported in A. scherzerianum (Haborne, 1967).

2.6 Genetics of anthurium spathe color

Using a visual method of scoring, the genetics of spathe color have been studied over a number of years in Hawaii. Kamemoto and Nakasone (1955) reported that whites are true breeding, that a cross of white and red produces all-pink progeny, and crosses between pink and white produce a 1:1 segregation of pink and white. In 1963 they reported that three crosses of red x white had progenies which segregated into red and coral pink in a 1:1 ratio (Kamemoto and Nakasone, 1963). A cross of red x red gave all red but a red which was selfed gave a 3:1 ratio of red to orange. An orange which was selfed gave only orange. Orange crossed to red gave two different results: one in which all were red and one which gave a 1:1 ratio of orange to red. They concluded that a multiple allelic system was involved in which red was dominant to orange and both were dominant to white. This system of inheritance seemed to also explain the cross reported in 1955 between red and white which gave an all-pink progeny.

The multiple allelic scheme of inheritance of spathe color, also involving interacting modifying genes was discussed in a 1968 report

(Kamemoto et al.). However, in a report on interspecific hybridization in 1977, Kamemoto and Sheffer discussed findings which suggested complementary gene action with at least two different genes.

III. MATERIALS AND METHODS

3.1 Plant material

The anthurium plants used in this study are part of the University of Hawaii collection. Some of these plants are clones collected from growers in Hawaii. Others are advanced selections obtained in the breeding program and still others are cultivars named and released by the University of Hawaii.

Three progenies were anlayzed. The first (457) was a cross between UH 515 and 'Marian Seefurth'. UH 515 is a dark coral selection of a cross between 'Fukano' and 'Manoa Mist'. It has been used in a number of crosses previously and the segregations by color of offspring of the different crosses are shown in Table 1.

'Marian Seefurth' is a University of Hawaii pink release which resulted from a cross between 'Haga White' and a pink accession (Kamemoto and Nakasone, 1963; Kamemoto et al., 1977). It, too, has been used in many crosses and the segregations by color are shown in Table 1.

The second progeny (464) analyzed was from a cross between UH 507 and A 360-63. UH 507 is a pink selection which was obtained from a cross between 'Marian Seefurth' and 'Kansako No. 1', a red accession. It has been used in a number of crosses, and the segregations by color are shown in Table 2.

A 360-63 is an orange accession which is spotted. No data is available on progeny of other crosses made using this plant.

The third progeny (435) analyzed was from a cross between 'Manoa Mist' and A 360-88. 'Manoa Mist' is a white University of Hawaii release which was obtained from a cross between 'Uniwai' and 'Marian

Table 1. Segregation by color of offspring of crosses with UH 515 or 'Marian Seefurth' as one parent.*

Cross	Color†												
	DR	R	LR	DP	P	O	LO	DC	C	LC	VLC	WT	W
UH 188 (C) x UH 515 (DC)						19	5	14	29	3	1	4	13
Manoa Mist (W) x UH 515 (DC)						2		5	10	2	2	2	3
Marian Seefurth (P) x DeWeese (W)		7	5		14				20			11	32
DeWeese (W) x Marian Seefurth (P)		3	17		8				19			16	30
Marian Seefurth (P) x Abe (P)		3	13		11				10			7	2
Marian Seefurth (P) x UH 17 (P)		4	6		39				7			7	10
Marian Seefurth (P) x Hirose (P)		31	4						6			4	2
UH 16 (P) x Marian Seefurth (P)		9	11	25					18			8	10
Marian Seefurth (P) x Kaumana (DR)	2	48	7		9		1		17				
Marian Seefurth (P) x Kansako (R)		43	8		5		1	2	19				
Marian Seefurth (P) x Nitta (O)		16	2		5	4		1	33				
Marian Seefurth (P) x UH 39 (C)		7	5		7			1	5			3	2

* Data from unpublished work, H. Kamemoto

† Key: DR = dark red, R = red, LR = light red, DP = dark pink, O = orange, LO = light orange, DC = dark coral, C = coral, LC = light coral, VLC = very light coral, WT = white tinged, W = white.

Table 2. Segregation by color of offspring of crosses with UH 507, A 360-88 or 'Manoa Mist' as one parent.*

Cross	Color†											
	DR	R	LR	DP	P	O	LO	DC	C	LC	VLC	WT	W
UH 507 (P) x UH 529 (Bright R)		41	19	2	12				1	1		2	4
UH 507 (P) x Manoa Mist (W)		3	3		5				10	4		2	16
UH 507 (P) x UH 515 (DC)		23	11		5	3	1	9	21	3		5	8
UH 507 (P) x A 360-88 (R)		11	20		14				7	8		2	10
DeWeese (W) x MM (W)												1	10
Manoa Mist (W) x A 220-2 (W)												5	53
Kanda (P) x Manoa Mist (W)					16	1			28			10	36
Hirose (P) x Manoa Mist (W)		33	9			5		1	5			27	3
UH 16 (P) x Manoa Mist (W)		1	4		2				7			4	7
Manoa Mist (W) x UH 186 (P)		13			18	2	2	1	17			17	29
Calypso (P) x MM (W)			3		2			1	6			9	1

* Data from unpublished work, H. Kamemoto.
† Key: DR = dark red, R = red, LR = light red, DP = dark pink, O = orange, LO = light orange, DC = dark coral, C = coral, LC = light coral, VLC = very light coral, WT = white tinged, W = white.

Seefurth'. 'Uniwai' was derived from a cross between 'Haga White' and a white accession. It has been used in numerous crosses and found to breed true for white. Segregation by color of the offspring of crosses with 'Manoa Mist' as one parent is shown in Table 2.

A 360-88 is a red spotted accession. Data on a cross with UH 507 are included in Table 2.

In this study the terms red and pink refer to colors corresponding to the red groups designated 40-55 in the Royal Horticultural Society Colour Chart. The terms orange and coral refer to colors corresponding to the orange and red groups designated 27-39 in the Royal Horticultural Society Colour Chart. The term orange group refers to dark orange, orange, light orange, dark coral, coral and light coral. The term red group refers to dark red, bright red, red, light red and pink.

3.2 Anthocyanin identification

Isolation and purification. Freshly harvested spathes were shredded and steeped in methanolic hydrochloric acid (MeOH-HCl) 0.1% under refrigeration for 24 hours. The volume was reduced under vacuum evaporation and the extract stored at 0°C.

Preparative chromatography was done using plates coated with 0.5 mm microcrystalline cellulose. The plates were prepared in the following manner. The 20 x 20 cm glass plates were thoroughly cleaned with detergent and hot water, dried and placed on the frame. The glass surface was washed with ethanol followed by acetone. Seven and one-half grams Microcrystalline Cellulose 'Baker TLC' and 50 ml de-ionized water were put into a blender for 60 seconds and immediately poured into the applicator boat adjusted to 0.5 mm discharge thickness. The boat

was slowly pulled across the plates in a smooth, even motion. This quantity was adequate for the 6 plates which could be fit on the frame. The plates were set on a very flat surface for drying which was accomplished in 24 hours.

Using a streak applicator, the concentrated extract was applied in a narrow band. Up to 3.75 ml of extract could be applied in a reasonable time when the applications were simultaneously blow-dried with air or N_2.

The plates were developed in a buffered acetonitrile system originally described for paper chromatography of phenolic compounds (Krishnamurty and Krishnaswami, 1975). Ammonium acetate 1 M was taken to pH 4.0 with acetic acid 1 M. It was then diluted to 0.1 M and the solvent system Buff CH_3CN (acetonitrile: 0.1 M ammonium acetate pH 4.0 7:3 vol/vol) was prepared. Development time for the plates was ca. 45 minutes.

After drying the plates for about 1.5 hours each cyanic band was scraped off the plate and the resultant powder pooled and eluted in MeOH-HCl 0.01% overnight and filtered, after which the volume was reduced under vacuum evaporation.

Purification of the isolated pigments was done using the solvent system BuHCl (n-butanol - 2N HCl 1:1 vol/vol). The purified pigment band was again eluted in MeOH-HCl 0.01%, filtered, and concentrated by evaporation under vacuum.

Partial acid hydrolysis. About 2 mg pigment in 1 ml 2N HCl was refluxed at 100°C. Aliquots were spotted on commercially precoated cellulose plates at 0, 5, 10, 20, 30, 40 and 60 minutes and developed

in AWH (glacial acetic acid-water-concentrated HCl 15:82:3 vol/vol).

Complete acid hydrolysis. To 5 mg pigment in a screw cap vial, 1 ml 2N HCl was added, flushed with N_2 and hydrolyzed at 100°C for 1 hour. The aglycone was extracted with 1 ml amyl alcohol and dried under N_2. The aqueous layer which was to be analyzed for sugar was first treated with ca. 3 ml di-n-octyl-methylamine and then washed with $CHCl_3$. The clear aqueous phase was dried under N_2.

Gas chromatography of sugars. The sugars in the hydrosylate were silylated with 50 µl Tri-Sil Concentrate and 1 ml anhydrous pyridine (prepared by placing one pellet KOH in pyridine for 4 hours), shaken and let stand at room temperature for 5 minutes. Sugars used as standards were silylated in like manner, using 5 mg of each sugar. A Bendix Series 2500 Gas Chromatograph with a 6 foot (1.8 m) Supelco 1% SE-30 column was used for the qualitative analysis.

Spectral measurements. The absorption spectra were measured on a Unicam SP 1800 Ultraviolet Spectrophotometer. The anthocyanins and anthocyanidins were dissolved in MeOH HCl 0.01%. The $AlCl_3$ shift was measured after adding three drops of 5% $AlCl_3$ in 95% ethanol.

Analytic chromatography. Co-chromatography of glycosides and aglycones with authentic pigments was done on commercially pre-coated plates (0.25 mm MN300 Cellulose Analtech Inc.) using the following solvent systems: Buff CH_3CN, BuHCl, BAW (n-butanol-glacial acetic acid-water 4:1:5 vol/vol), 1% HCl (water-concentrated HCl 97:3 vol/vol), AWH (glacial acetic acid-water-concentrated HCl 15:82:3 vol/vol), and Formic (formic acid-concentrated HCl-water 5:2:3 vol/vol).

Authentic pigments. Authentic pelargonidin and cyanidin were obtained by acid hydrolysis of pelargonidin chloride (Lot-16569-A) and cyanidin 3-,5-diglucoside (Lot-16165-A) respectively; both were purchased from ICN Pharmaceuticals. Pelargonidin and cyanidin 3-monoglucoside were isolated from the fruit of Fragaria (Lukton et al., 1955; Sondheimer and Karash, 1956). Pelargonidin 3-rutinoside was isolated from the spathe of Anthurium scherzerianum (Harborne, 1967) and cyanidin 3-rutinoside was obtained from petals of Nicotiana tabacum (Harborne, 1967). The 3-rutinosides of both cyanidin and pelargonidin are also readily available from the bracts of Euphorbia pulcerrima (Asen, 1958).

3.3 Noncyanic flavonoid characterization

Array of flavonoids. Freshly harvested spathes of a white anthurium clone were shredded, steeped in methanol and put on a shaker at room temperature for 48 hours according to the method used by Giannasi (1975). The extract was concentrated under vacuum and heavily applied in a spot ca. 0.5 cm in diameter on six sheets of 46 x 54 cm Whatman 3MM paper which had been washed with .02% oxalic acid and water and dried thoroughly. Two-dimensional descending chromatography was run using TEA (tert-butanol-glacial acetic acid-distilled water 3:1:1 vol/vol) in the first dimension and HoAc (distilled water-glacial acetic acid 85:15 vol/vol) in the second dimension.

Ultraviolet characterization. All the spots were observed under ultraviolet irradiation before and after fuming with ammonia. The chromatogram was fumed with ammonia by placing it in a sealed plastic bag with an open container of NH_4OH for 45 minutes and subsequently

viewed under ultraviolet irradiation.

All the major spots were cut out, cut into strips and eluted in methanol at room temperature for 24 hours. The eluate was concentrated by evaporation under vacuum.

Spectra of each spot were obtained. There was only one spot which had maximum absorption at 330 nm. All others had absorption maxima at 210-230 and 270-284 nm. Since flavones characteristically have maximum absorption at 330-350 nm, and flavones would probably have a greater influence on color than the shorter wavelength absorbing flavonoids, this particular pigment was selected for further chemical characterization. Judging from spot size on the chromatogram, this pigment was also the major flavonoid.

Isolation. Preparative quantities of pigment were isolated from the TLC plates used to isolate anthocyanins which had been developed in CH_3CN. The entire area above the anthocyanin bands was scraped off the plates and eluted in methanol. The eluate was evaporated under vacuum and the concentrate applied as a band to Whatman 3MM paper and developed in BAW by descending chromatography. The band was located and outlined under ultraviolet irradiation, cut into strips and eluted in methanol at room temperature for 24 hours, evaporated under vacuum to dryness, and then taken up in methanol.

Authentic pigment. Acacetin 7-neohesperidoside was isolated from the rinds of the immature fruitof Fortunella japonica (Matsuno, 1958).

Analytic chromatography. Co-chromatography of the glycoside with the authentic pigment was done on Whatman 1 MM paper in descending chromatography in the following solvent systems: Water, HoAc, BAW and

TBA.

Spectroscopic analysis. Spectra were obtained of the purified pigment in methanol, and after addition of the following reagents: 5% aluminum chloride in methanol ($AlCl_3$), saturated solution of sodium acetate (NaOAc), boric acid added to sodium acetate to saturation (H_3BO_3) and sodium methoxide (NaOMe) prepared by dissolving 2.5 g Na in 100 ml MeOH according to the methods described by Mabry, et al. (1970).

Partial acid hydrolysis. Methanol was added to 5 ml of the concentrated purified pigment to a volume of 30 ml to which 2-3 drops of concentrated HCl were added. This solution was refluxed at 100°C for 3 hours during which 4 ml aliquots were removed every half hour and spotted on Whatman 1 MM paper and developed in the solvent systems BAW and Water. The remaining part of each aliquot was taken to dryness and taken up in methanol for spectral analysis.

3.4 Quantification of anthocyanins and the flavone

Spathes were harvested within 2 weeks of the time when they unfurled and/or at the time when there was a change in spadix color to one half the length of the spadix. Change in spadix color occurs acropetally and harvest of the inflorescence as a cut flower is generally done when the change approaches the halfway point of the spadix. This feature was used as an index to keep age of spathe uniform.

One gram of shredded fresh spathe was steeped in 200 ml of MeOH HCl 0.1% under refrigeration for 24 hours. The volume was reduced under vacuum evaporation, adjusted to 3 ml, and stored at 0°C. Within 4 weeks the extract was analyzed. Ten µl of the extract was brought to 10 ml volume with methanol, and the OD (optical density) value at 330 nm was

obtained. Using 750 µl, individual anthocyanin bands were obtained by TLC using Buff CH_3CN as the solvent system. The magenta band was eluted in 50 ml methanol, kept in the refrigerator for 24 hours, filtered, and the OD value obtained at 530 nm. The orange band was eluted in 10 ml methanol, kept in the refrigerator for 24 hours, filtered and the OD value obtained at 520 nm.

These OD values were converted to total OD value per g fresh weight using the method of Fuleki and Francis (1968). Their formula for calculating mg of anthocyanin for 100 g of cranberries was as follows:

Total anthocyanin in mgs per 100 g = OD X DV X 100/SV X TEV/CrW X 1/98.2 where

OD = the absorbancy reading on the diluted sample (1 cm cell)

CV = Diluted Volume or the volume in mls of the diluted extract prepared for the OD measurement.

VF = Volume Factor which corrects for the difference in size between 100 ml, the Original Volume (OV) for which the calculation is made and that of the Sample Volume (SV) or the volume of extract used for the absorbancy measurement.

VF = OV/SV = 100/SV

TEV = Total Extract Volume. The total volume of the extract in mls obtained from the Cr sample used for extraction.

CrW = Cranberry Weight (or the weight of the Cr) in gms used for extraction.

Since no accurate extinction values for the particular anthocyanins identified in this study were found, the calculations made for each sample were for total OD per g fresh weight of spathe.

The calculations used in this report were done using the following values:

OD = absorbance obtained using 1 cm cell

DV = 10 ml (for 330 nm reading)
50 ml (for 530 nm reading)
10 ml (for 520 nm reading)

VF = 1/.1 where 1 is an arbitrary number (for 330 nm reading)
1/.75 (for 530 nm reading)
1/.75 (for 520 nm reading)

TEV = 3 ml

CrW = 1 gram

The entire calculation was reduced to the following:

For the 330 mm reading:

OD X 10 ml X 1/.1 X 3/1 = OD X 300

For the 530 nm reading:

OD X 50 ml X 1/.75 X 3/1 = OD X 200

For the 520 nm reading:

OD X 10 ml X 1/.75 X 3/1 = OD X 40

These values are used in this entire report because a simple multiplication step using accurate extinction coefficients will provide the mg per g fresh weight values. OD values presented as total OD per g fresh weight result in a uniform method of reporting so that comparisons can be made directly with other values presented in terms of OD per g fresh weight.

The quantitative reading for the flavone was taken directly from the unpurified extract because no other pigment present in appreciable quantity has a primary absorption maximum in that vicinity. The anthocyanins have primary peaks at about 270 and somewhere above 500, so they should not interfere with the 330 nm absorption. Other flavonoids isolated by 2-dimensional chromatography all had major peaks at 210-230

and minor peaks at 270-284 nm. These values are at least 100 nm away from the 330 absorption of the flavone so that it is reasonable to assume that the absorption value taken at 330 nm primarily reflects the concentration of the flavone which was characterized in this study. Subsidiary peaks are known to occur in this area for flavanone, flavanonol and isoflavonoid; however, none of any notable intensity were found in spectra of the other flavonoids isolated by 2-dimensional chromatography.

The reliability of the methodology for extracting, isolating and measuring each band was tested by taking readings of 4 aliquots of the same extract. Readings were also taken of individual pigments after they were chromatographically isolated. Readings were also taken using extracts of spathes from 4 different plants of one clone.

To get a measure of the variability occurring at different seasons, spathes of one clone were tested at different times during the year. Results of these tests are in Appendix I.

IV. RESULTS AND DISCUSSION

4.1 Identification of the flavonoids

Anthocyanins. Two cyanic bands were isolated from the cultivar 'Kaumana'. One band was an orange pigment and had a higher R_f in both the Buff CH_3CN and BuHCl solvent systems. The other band was a magenta pigment.

Partial acid hydrolysis of each of the isolated bands produced three spots, corresponding to the aglycone, the monoglycoside, and the diglycoside, indicating that both pigments were diglycosides. The aglycones were co-chromatographed in several solvent systems with pelargonidin and cyanidin standards. Spectra of the unknown aglycones matched those of authentic cyanidin and pelargonidin (Figure 3). Table 3 shows the R_f values and the spectral characteristics of these pigments. These results indicated that the orange pigment was a pelargonidin diglycoside and that the magenta pigment was a cyanidin diglycoside.

Gas chromatography of the silylated sugars, derivatives of those obtained after acid hydrolysis of each of the bands, indicated that the sugars in each case were glucose and rhamnose (Table 4, Figure 4). The two retention times for each sugar represent the alpha and beta anomeric forms which are expected in an aqueous biologic extract (Sweeley et al., 1963). The possibility of acylation was investigated by alkaline hydrolysis. Results were negative. Chromatographic behavior, dull appearance under ultraviolet illumination and spectral characteristics (Table 5) indicated that both pigments were 3-rhamnosylglucosides of cyanidin and pelargonidin. There was no

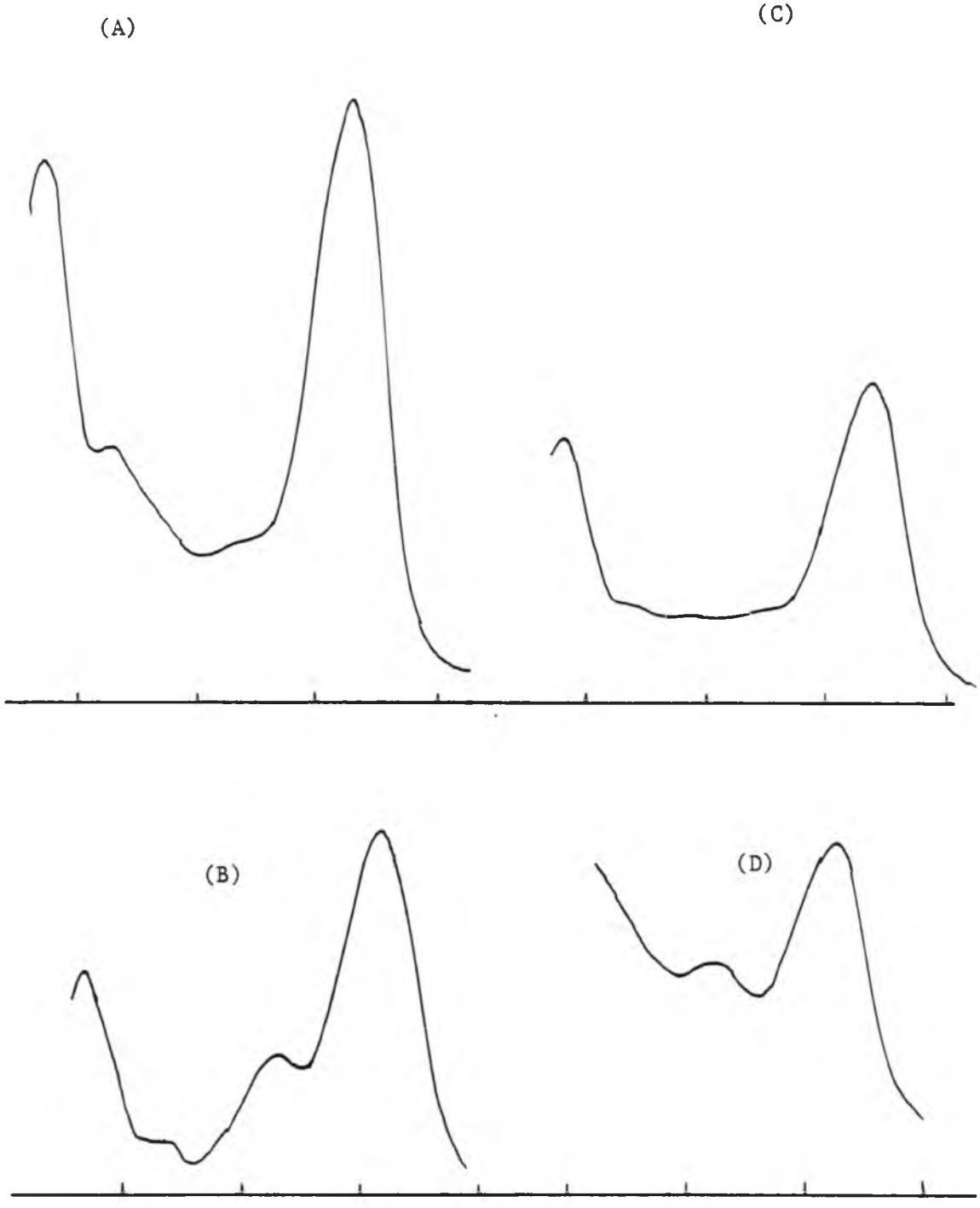


Figure 3. Spectra of (A) cyanidin, (B) pelargonidin, (C) magenta anthocyanidin and (D) orange anthocyanidin from A. andraeanum.

Table 3. R_f x 100 values on TLC and spectral measurements of anthocyanidins from Anthurium andraeanum.

Anthocyanidins	Buff CH_3CN	Forestal	Formic	BAW	Absorption max (nm)	% abs 440/vis max	$AlCl_3$ shift
Authentic pigments:							
Pelargonidin	80	79	33	92	520 (520, 270)	50 (39)*	-(-)
Cyanidin	59	63	21	78	536, 276 (535, 277)	28 (19)	+(+)
Isolated:							
Orange pigment	80	79	33	92	520	39	-
Magenta pigment	59	63	21	78	538, 274	23	+

* Values in parentheses are from Harborne, 1967.

Table 4. Retention times of silylated standards and the sugar residue of anthocyanin glycosides of Anthurium andraeanum obtained by gas chromatography.

Sugar	Retention times (min)*
Glucose	3.2; 5.5
Galactose	2.8; 3.3
Rhamnose	0.8; 1.0
Band I sugars	0.8; 1.0; 3.2; 5.5
Band II sugars	0.8; 1.0; 3.2; 5.5

* Each sugar has two peaks representing the alpha and beta anomeric forms.

Figure 4. Gas chromatogram of silylated sugar derivatives of sugars obtained by hydrolysis of both anthocyanins of A. andraeanum.

Table 5. Spectral measurements and R_f x 100 values on TLC of authentic and isolated anthocyanins from Anthurium andraeanum.

Anthocyanin	Buff CH_3CN	BAW	BuHCl	HCl 1%	AWH	Absorption max (nm)	% abs 440/vis max
Authentic pigments:							
Pelargonidin 3-rutinoside	61	47	40	25	58		
Cyanidin 3-rutinoside	54	40	32	17	47		
Pelargonidin 3-glucoside	59	47	41	13	40	512, 270	49 (38)
						(506, 270)*	
Cyanidin 3-glucoside	49	40	26	05	24	532, 254	37 (24)
						(523, 274)	
Isolated pigments:							
Orange band	61	48	42	26	57	512, 270	56
Magenta band	54	41	33	20	48	532, 280	40

* Values in parentheses are from Harborne, 1967.

indication that the rhamnosylglucoside was other than the common one, rutinoside, found in anthocyanins (Harborne, 1967). No attempt was made to determine the exact nature of the linkage between the sugar moieties.

Less than 45 minutes were required to develop 20 x 20 cm chromatograms coated with 0.5 nm microcrystalline cellulose or the commercially pre-coated plates in the buffered acetonitrile system. This is less than half the time required for developing paper in this system (Krishnamurty and Krishnaswami, 1975). The plates were run without pre-equilibration and gave excellent resolution of the two pigments. This method was proved quite satisfactory for genetic investigations which involve large numbers of samples because numerous plates can quickly be processed in this manner.

Noncyanic flavonoids. A total of 15 spots were observed on the two-dimensional descending paper chromatograms (Figure 5). The chromatographic, UV and spectral characteristics of these spots are presented in Table 6. The spectrum of each spot is presented in Appendix II.

Since flavonols, chalcones and aurones have spectral maxima over 350 nm, it was concluded that no flavonols, chalcones or aurones were present in the sample tested. Spot No. 7, which has absorption maxima of 330 and 274 nm is the only one which falls within the range of maxima for flavones (330-350 nm).

Spot No. 8 may be a cinnamic acid because of the aqua color in the UV after fuming with ammonia. The cinnamic acids are fairly ubiquitous in vegetative parts and are colorless in the visible light, bluish in UV and green in the UV after fuming with ammonia (Swain, 1976). The

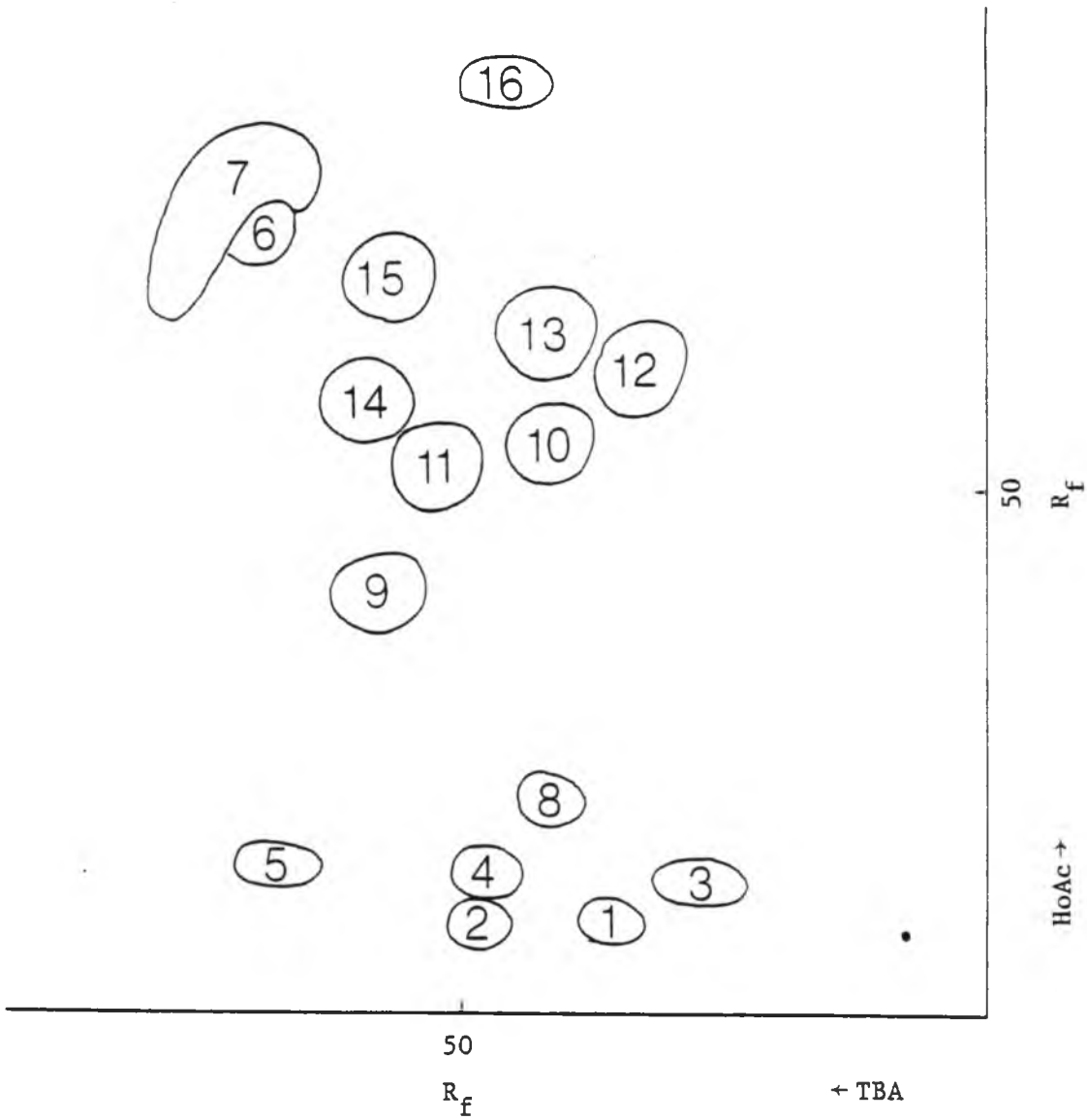


Figure 5. Diagrammatic representation of two-dimensional paper chromatogram depicting relative positions of 16 spots from methanolic extract of the spathe of A. andraeanum.

Table 6. Chromatographic, UV and spectral characteristics of spots from a methanolic extract of anthurium spathes.

Spot. No.	R_f x 100 TBA	R_f x 100 HoAC	Vis	UV	UV/NH_3	Spectral Maxima
1	34	0	pY	O	O	214, 236
2	49	0	C	pO	faint	230, 260
3	29	5	pY	FlY	Y	230, 270
4	47	6	C	FlBl	pBl	230, 260
5	70	7	C	pY	faint	218, 270
6	72	76	C	FlY	FlY	216, 278
7	75	82	pY	Q	Q	274, 330
8	41	22	C	C	Aqua	
9	60	37	C	FlY	FlY or G	210, 270
10	41	54	pY	pY	pO	210, 282
11	53	51	C	Q	Bl	208, 284
12	30	63	Y	slQ	pO	208, 280
13	41	66	Y	YO	O	210, 284
14	61	58	C	Q	faint	210, 270
15	58	72	C	slQ	slQ	210, 270
16	47	94	C	pY	faint	210, ?

Key: C = colorless, pY = pale yellow, Y = yellow, FlY = fluorescent yellow, FlBl = fluorescent blue, Q = quenching, slQ = slightly quenching, YO = yellow orange, pBl = pale blue, G = green, pO = pale orange.

majority of the spots have maxima which fall within the range of the flavanones and flavanonols 275-290 nm. Two spots have maxima which fall within the range of the isoflavones. No attempt was made to place these spots into classes of compounds.

Spot No. 7 was subjected to further scrutiny to characterize the compound. The spectrum taken in MeOH (Figure 7) shows two well-defined peaks with a Band II maximum of 274 nm and a Band I maximum of 330 nm. A single well-defined Band I maximum in the range 304-350 is characteristic of flavones (Markham et al., 1970; Jurd, 1962). A single Band II peak indicates that there is only a 4' substituent (Markham et al., 1970; Jurd, 1962) in contrast to 3', 4' substitution, for example.

Addition of $AlCl_3$ (Figure 6) resulted in a splitting of Band I into a peak and shoulder with a bathochromic shift of 48 nm and a splitting of Band II into two peaks with a Band IIa bathochromic shift of 32 nm. A free 5-OH on a flavone forms stable yellow complexes with aluminum resulting in moderate bathochromic shifts of Bands I and II, each of the peaks characteristically having two peaks or inflections. The Band I shift is 20-45 nm (Jurd, 1962). A free 5-OH is thus indicated. A free B ring dihydroxyl group would have resulted in a greater shift (60 nm) without splitting of the peak. Therefore it is concluded that no free B ring dihydroxyl group is present. This conclusion is supported by the presence of a single Band II peak of the original MeOH spectrum which indicates only a 4' substituent.

Addition of NaOAc (Figure 7) resulted in no shift of either peak, indicating that the 7-OH is substituted and thus protected. A free 7-OH would have resulted in an 8-20 nm shift of Band II (Jurd, 1962) and/or

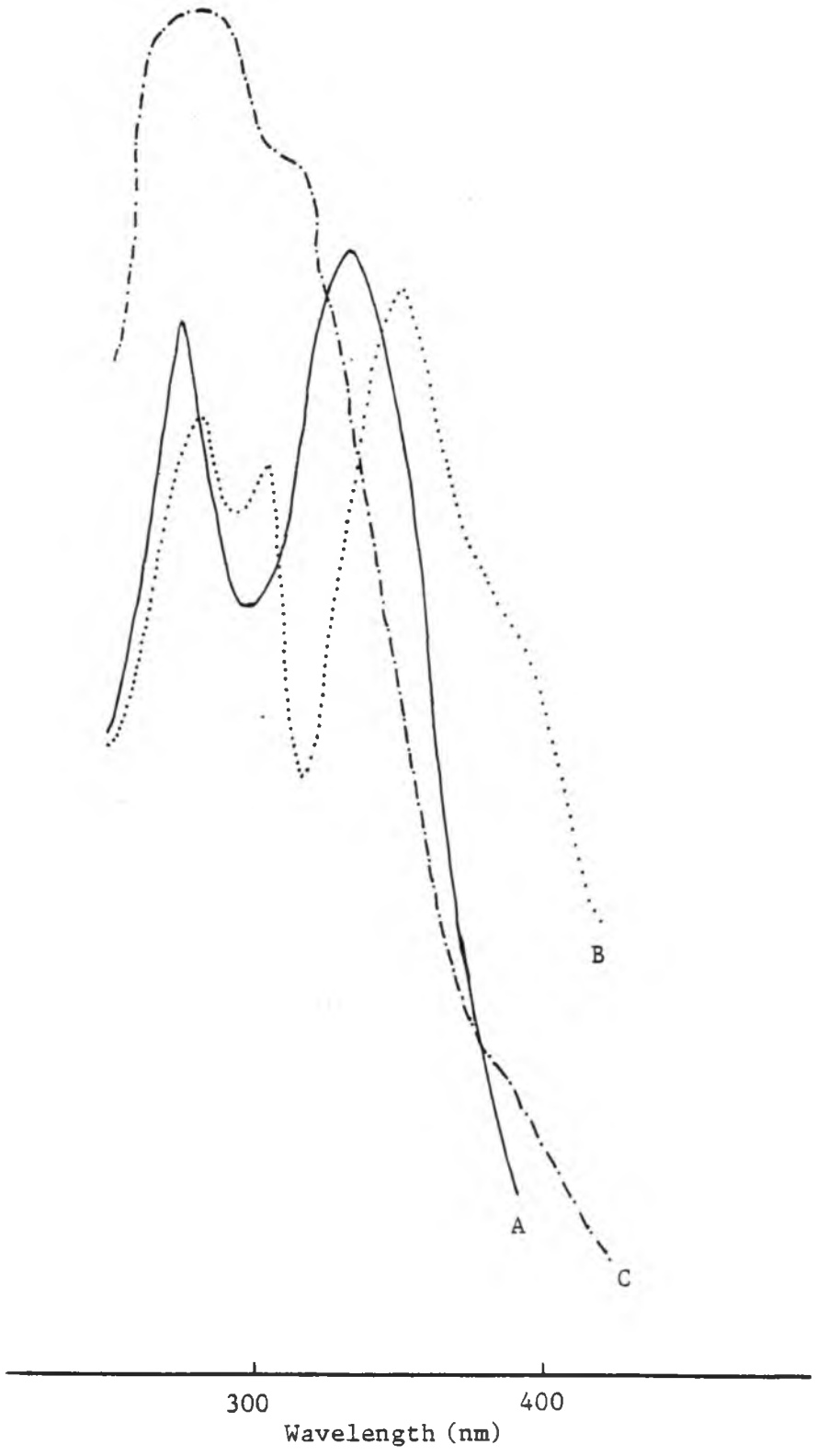


Figure 6. Spectra of Spot No. 7 in MeOH (A), 274, 330 nm; $AlCl_3$ (B), 282, 306 nm; and NaOMe (C), 290 nm.

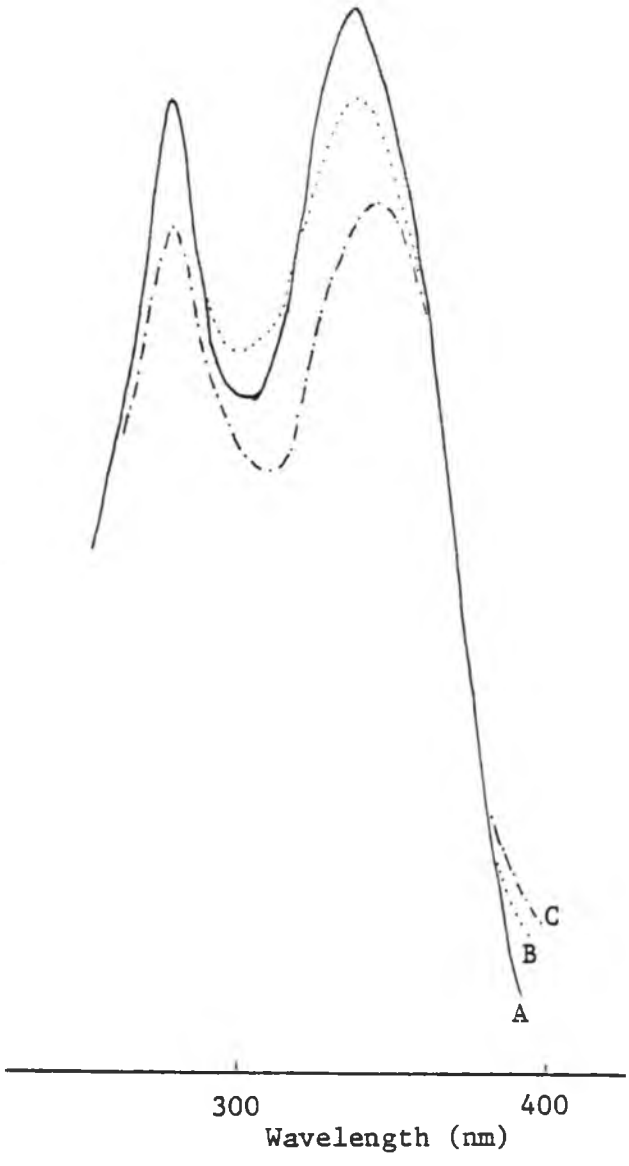


Figure 7. Spectra of Spot No. 7 in MeOH (A) 274, 330 nm; NaOAc (B), 274, 330 nm; and H_3BO_3 (C), 274, 338 nm.

collapse of Band I.

Boric acid added to the point of saturation to the NaOAc solution resulted in a Band I bathochromic shift of 8 nm (Figure 7). A larger shift (12-13 nm) would be expected with a free B-ring dihydroxyl group. A small shift (5-10 nm) such as this may be indicative of 6,7 or 7,8 dihydroxyl groups. This result confirms the inference made from the results of the addition of $AlCl_3$, i.e., there is no free ortho-dihydroxyl group in ring B. The results of the addition of NaOAc contradict the possibility of the presence of a free 6,7 or 7,8 dihydroxyl group.

The addition of NaOMe resulted in a collapse of the Band I peak, suggesting that there is a 4' substitution (Figure 6). This corroborates the inference made from the dark absorbing appearance of the spot under ultraviolet irradiation before and after fuming with ammonia that there is 4' substitution (Giannasi, 1975).

The spectral characteristics of the unknown compounds were compared with those which have been published (Markham et al., 1970; Giannasi, 1975). The flavone acacetin 7-rutinoside and 7-glucoside have spectral characteristics very much like those of the unknown compound, Spot No. 7 (Figure 8); however, the chromatographic behavior of the unknown deviated considerably from that of acacetin 7-rutinoside and acacetin 7-glucoside (Table 7).

Chromatographic behavior of other acacetin 7-glycosides was reviewed in the literature. Of those with published R_f values, none were found to possess the unusual characteristics of very high mobility in both aqueous and alcoholic solvent systems although acacetin

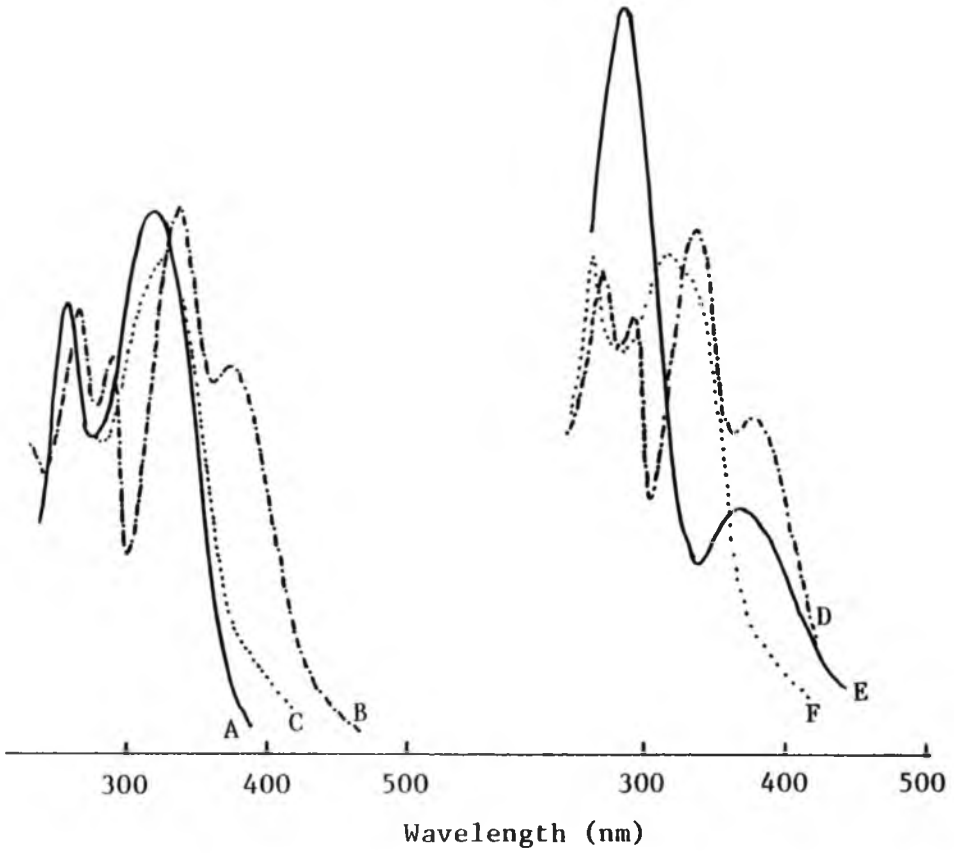


Figure 8. Spectra of acaetin 7-rutinoside. (A) MeOH - 325, 267; (B)$AlCl_3$ - 381, 345, 297, 279; (C) NaOAc/H_3BO_3 - 330, 266; (D) $AlCl_3$/HCl - 380, 336, 296, 272; (E) NaOMe - 370, 295, 285; (F) NaOAc - 320, 268. From Giannasi, 1975.

Table 7. R_f x 100 of the unknown compound and some flavone glycosides.

Compound	UV & UV/NH3	TBA	Water	BAW	HoAc
Acacetin 7-rutinoside *	purple	65			53
Acacetin 7-glucoside *	deep purple	58			27
Acacetin †	deep purple	90			11
Spot No. 7, Unknown	purple	73	72	68	81
Acacetin 7-neohesperidoside	purple	71	69	68	78

* From Giannasi (1975).
† From Mabry et al. (1970).

7-rutinoside has fairly high R_f's in both TBA and HoAc.

Because of the very similar spectral characteristics between the unknown Spot No. 7 and acacetin 7-rutinoside, and because no R_f values of the 7-neohesperidoside were found in the literature, the chromatographic behavior of acacetin 7-neohesperidoside, which differs from acacetin 7-rutinoside only in the linkage of glucose to rhamnose, was determined. Rutinose has an alpha 1 → 6 linkage and neohesperidose an alpha 1 → 2 linkage.

Acacetin 7-neohesperidoside was isolated from Fortunella japonica by preparative paper chromatography using BAW. R_f values are presented in Table 7. They are very similar to those of the unknown compound Spot No. 7.

The appearance of acacetin 7-neohesperidoside under ultraviolet illumination before and after fuming with ammonia was also like that of the unknown compound.

Spectral characteristics of acacetin 7-neohesperidoside were obtained (Figures 9, 19). They are not unlike those of the unknown compound except for the unexpected response to NaOAc which normally occurs with a free 7-OH.

Partial acid hydrolysis was attempted without definitive results because of the insufficient amount of pigment on hand.

On the basis of the data obtained, the unknown compound is very likely the flavone acacetin with a sugar substituent at position 7.

4.2 Pigment characteristics of spathe color

Using the data obtained from analyses of cultivars and advanced selections (Appendix III), several observations were made. The

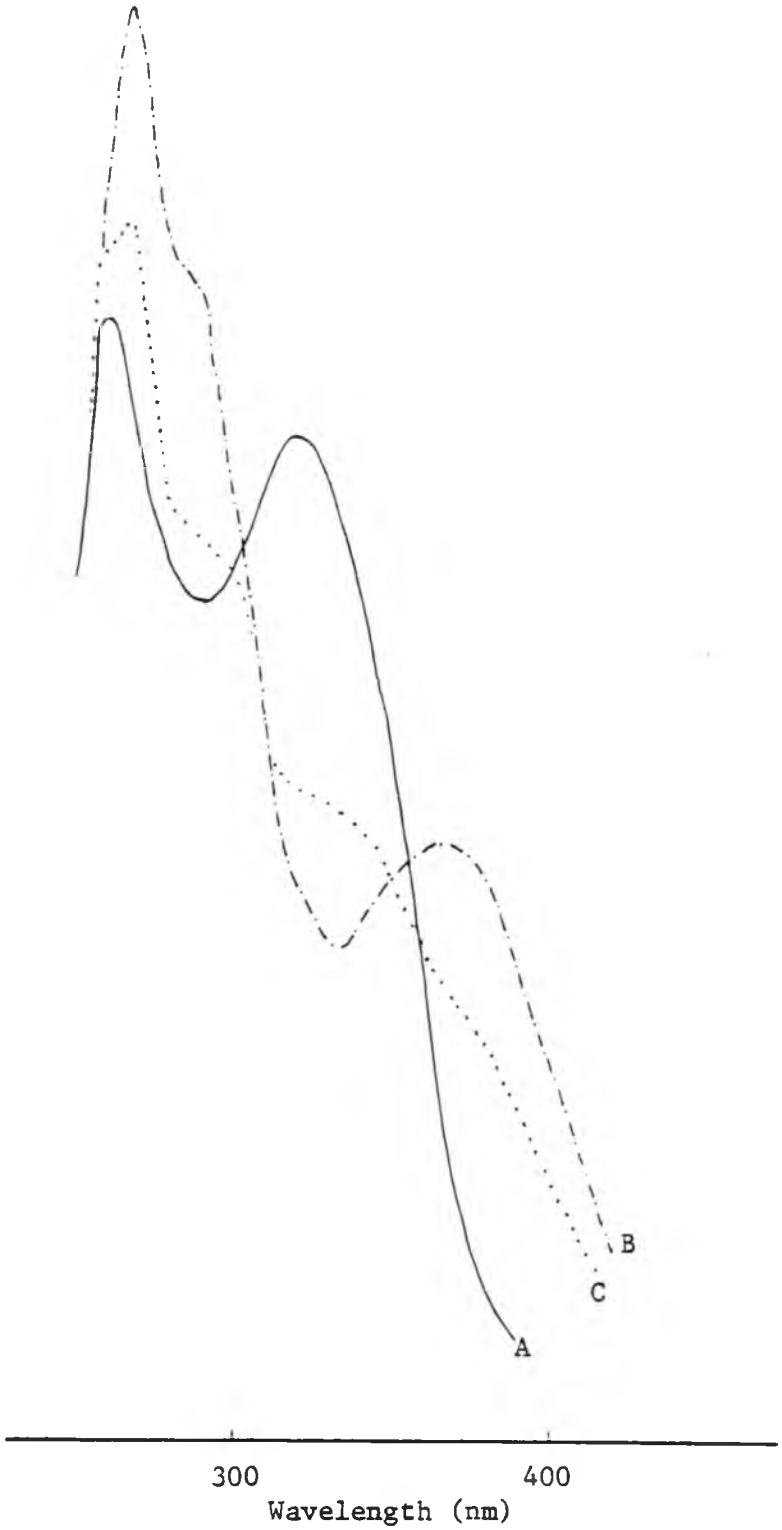


Figure 9. Spectra of acacetin 7-neohesperidoside in MeOH (A), 274, 330 nm; NaOMe (B), 282, 374 nm; and NaOAc (C), 280 nm.

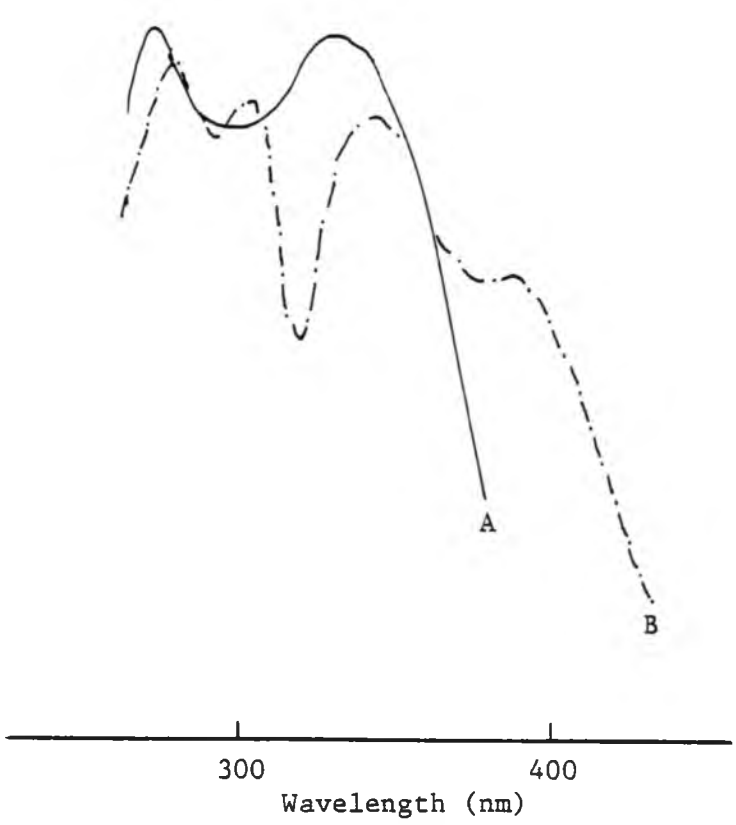


Figure 10. Spectra of acacetin 7-neohesperidose in MeOH (A), 274, 330 nm; and $AlCl_3$ (B), 280, 304, 340, 382.

concentration of anthocyanins was found to be the major factor influencing the color of spathes. The dark reds had high concentrations of cyanin and this concentration decreased substantially in the bright reds (Table 8). The concentration continued to decrease into the reds, dark pinks and pinks until none was detected in the whites. There was a variable but small amount of this pigment in the different shades of coral and orange.

The concentration of pelargonin was of moderate intensity in the dark reds and appears to increase in the bright reds. There were generally lesser amounts found in the reds, pinks and corals. The highest concentrations were found in the orange spathes.

The concentration of the flavone was fairly high in all colors. The variability in concentration did not seem to be directly related to the shades of color as was the case with the anthocyanins.

In red-hued spathes both pelargonin and cyanin always co-occurred in all the clones examined. In this red group the concentration of the cyanin pigment was always greater than that of the pelargonin pigment. This relationship varied in the orange-hued spathes. In some orange-hued spathes, both pigments co-occurred with the pelargonin pigment having the higher concentration. Presence of the cyanin in concentrations below that of the co-occurring pelargonin results in no detraction from the apparent orange hue. In others, no cyanidin 3-rhamnosylglucoside was detected.

The "rose opal" and "coral pink" described by Kamemoto and Nakasone (1963) have been clearly differentiated biochemically. The "rose opal" in this study is termed pink and the "coral pink" is termed light coral.

Table 8. Ranges and means of the concentrations of the flavone, cyanin and pelargonin expressed as OD per gram fresh weight of different color classes of spathes of A. andraeanum.

Color Class	No. of Clones	Flavone (nm)		Cyanin (nm)		Pelargonin (nm)	
		Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Dark Red	13	216-420	315 ± 17.1	216-504	317 ± 24.0	2.9-27	15 ± 7.7
Bright Red	10	180-360	266 ± 19.1	134-196	161 ± 6.7	4.8-50	22 ± 4.5
Red	6	147-261	201 ± 19.1	58-154	103 ± 16.2	1.6-12	7 ± 1.5
Light Red	3	153-426	247 ± 89.5	23-88	58 ± 19.0	4.5-14.4	10 ± 3.0
Dark Pink	4	303-345	326 ± 10.6	18-60	39 ± 9.3	0.5-10.4	4 ± 2.2
Pink	4	108-257	215 ± 35.8	18-34	25 ± 3.4	0.7-3.6	2 ± 0.7
White	1	330		0		0	
Light Coral	1	360		0		1	
Dark Coral or Light Orange	3	198-246	216 ± 15.0	0-1.6	1 ± 0.5	4-13.6	8 ± 2.8
Orange	4	210-372	278 ± 34.3	0-4.8	2 ± 1.1	15.2-66.4	46 ± 12.0

In the pink the dominant pigment is cyanin and in the coral, the dominant pigment is pelargonin. The corals may have the pelargonin pigment as the only detectable pigment whereas the pinks have both pelargonin and cyanin.

4.3 Qualitative inheritance of pigments

A multiple allelic system of inheritance for the control of spathe color was suggested by Kamemoto and Nakasone in 1963. In this system the alleles $\underline{R}^{R}$, $\underline{R}^{O}$ and $\underline{r}$ were responsible for red, orange and white respectively.

A series of crosses between white and pink has been made since 1963. The progenies always segregated into a red group (pink to red), an orange group (coral to orange) and white. It is difficult to envision this kind of segregation arising with only one locus involved. If the white parent were homozygous recessive ($\underline{rr}$) and the pink heterozygous for the red and orange allele ($\underline{R}^{R}\ \underline{R}^{O}$) then the expected segregation would involve only the red and orange groups. Sheffer and Kamemoto (1977) discussed findings from interspecific hybridizations which suggested the involvement of at least two genes. The existence of both anthocyanins, cyanidin and pelargonidin glycosides, in pink to red spathes further indicates that two major genes are responsible for anthocyanin production. It is therefore proposed that the gene $\underline{M}$ controls production of cyanidin 3-rhamnosylglucoside and that the gene $\underline{O}$ controls production of pelargonidin 3-rhamnosylglucoside.

Since pink to red spathes contain both pigments, and some coral to orange spathes only pelargonidin, the $\underline{O}$ locus exhibits recessive epistasis over the $\underline{M}$ locus so that the dominant allele must be present

at the O locus in order for the dominant M gene to exhibit penetrance. Thus, the following genotypes would correspond to the following color groups. The double recessive mmoo would be white as would Mmoo or MMoo because of recessive epistasis. The genotypes for the red group would be MMOO, MmOo, MMOo and MmOO. Genotypes for the orange group would be mmOo and mmOO.

Table 9 gives the segregation of three progenies, Cross 457 (coral x pink), Cross 464 (pink x light orange) and Cross 435 (white x light red). In Cross 457 between a dark coral and pink, the parent genotypes are hypothesized to be mmOo and MmOo leading to an expected ratio of 3 red: 3 orange: 2 white. The red phenotypes would be comprised of the genotypes MmOO, MmOo; orange phenotypes would be comprised of the genotypes mmOO and mmOo; and white phenotypes would be comprised of the genotypes Mmoo and mmoo.

In Cross 464 between a pink and light orange, the parent genotypes are hypothesized to be like those of the parents in Cross 457 so that the expected phenotypic ratio would be the same, 3:3:2.

In Cross 435 between a white and a light red, the parent genotypes are hypothesized to be mmoo and MmOo leading to an expected phenotypic ratio of 1 red: 1 orange: 2 white. In this cross the genotype of the offspring corresponding to the red phenotype is MmOo; genotype corresponding to the orange phenotype is mmOo and the genotypes corresponding to the white phenotypes are Mmoo and mmoo.

The chi square results for the three crosses based on visual scoring support the hypothesized parent genotypes (Table 9). The hypothesized parent genotypes were further tested by analysis of progeny data of crosses that had been made in this laboratory which

Table 9. Segregation of offspring of the three crosses 457, 464 and 435 by color and pigment character.

Cross	Frequency	Phenotypic Character			χ^2	P
		Visually Scored*				
		Red Group	Orange Group	White Group		
457	Observed	26	33	21		
	Expected (3:3:2)	30	30	20	0.63	.90
464	Observed	37	29	15		
	Expected (3:3:2)	30.375	30.375	20.25	2.38	.50
435	Observed	19	15	39		
	Expected (1:1:2)	18	18	36	0.53	.90
		Chemically Scored				
		Cyanin + Pelargonin	Pelargonin	White		
457	Observed	44	15	21		
	Expected (3:3:2)	30	30	20	13.1	.01
464	Observed	56	10	15		
	Expected (3:3:2)	30.375	30.375	20.25	34.85	.001
435	Observed	23	10	39		
	Expected	18	18	36	4.43	.20

* Red Group includes red and pink; Orange Group includes orange and coral.

involved the parents (Tables 10 and 11). In 4 of the 17 crosses analyzed, the ratios established on the basis of the hypothesized parental genotypes were rejected; however, chi square results generally supported the hypothesized parental genotypes.

Chemical analyses of pigments of offspring from the three crosses, 457, 464 and 435 were also scored and placed in the following groups: (1) containing cyanin and pelargonin, (2) containing only pelargonin and (3) no anthocyanin detected (Table 9). The ratios on the biochemically scored progeny based on pigment content did not conform to those visually scored. The possible explanation for the discrepancy is discussed in section 4.4.

Results of an interspecific cross between A. andraeanum and A. roraimense suggest complementary gene action. The A. andraeanum cultivar 'Nitta' has an orange spathe and the A. roraimense (A 189) plant has a green spathe. The F_1 uniformly has red spathes, which upon cursory examination of R_f and spectra appear to have the same cyanin and pelargonin pigments that have been identified in red spathes of A. andraeanum. If 'Nitta' has the genotype mmOO and A 189, MMoo, then all offspring will have MmOo genotypes which phenotypically are red. It appears that the two-gene complementary system of inheritance is a viable explanation for the observed progeny spathe color phenotypic ratios.

4.4 Inheritance of quantitative variation of pigments

Anthocyanin concentration has a profound effect on spathe color, as we have seen in section 4.2. Parents and progenies of the three crosses 457, 464 and 435 were analyzed quantitatively for cyanin, pelargonin and the flavone. Results of these analyses are presented in Tables 12 and

Table 10. Segregation of offspring of crosses which have UH 515 or 'Marian Seefurth' as one of the parents.

Cross	Frequency	Phenotypic Character			χ^2	P
		Red Group	Orange Group	White Group		
UH 188 (C) x UH 515 (DC) (mmOo)* (mmOo)	Observed		71	17		
	Expected (3:1)		66	22	1.22	.30
Manoa Mist (W) x UH 515 (DC) (mmoo) (mmOo)	Observed		21	5		
	Expected (1:1)		13	13	8.66	.01
UH 507 (P) x UH 515 (DC) (MmOo) (mmOo)	Observed	39	37	13		
	Expected (3:3:2)	33.375	33.375	22.25	4.52	.20
Marian Seefurth (P) x DeWeese (W) (MmOo) (mmoo)	Observed	26	20	43		
	Expected (1:1:2)	22.25	22.25	44.5	.63	.90
Marian Seefurth (P) x Abe (P) (MmOo) (MmOo)	Observed	27	10	9		
	Expected (9:3:4)	25.875	8.625	11.5	.46	.90

Table 10. (Continued) Segregation of offspring of crosses which have UH 515 or 'Marian Seefurth' as one of the parents.

Cross	Frequency	Phenotypic Character			χ^2	P
		Red Group	Orange Group	White Group		
DeWeese (W) x Marian Seefurth (P) (mmoo) (MmOo)	Observed	28	19	46		
	Expected (1:1:2)	23.25	23.25	46.5	1.38	.70
Marian Seefurth (P) x UH 17 (P) (MmOo) (MmOo)	Observed	49	7	17		
	Expected (9:3:4)	41.06	13.69	18.25	4.18	.20
Marian Seefurth (P) x Hirose (P) (MmOo) (MmOo)	Observed	35	6	6		
	Expected (9:3:4)	26.45	8.8	11.75	5.40	.10
UH 16 (P) x Marian Seefurth (P) (MmOo) (MmOo)	Observed	45	18	18		
	Expected (9:3:4)	45.56	15.19	20.25	.5	.90

* Hypothesized genotypes.
Key: C = coral, DC = dark coral, W = white, P = pink.

Table 11. Segregation of offspring of crosses which have UH 507, A 360-88 or 'Manoa Mist' as one of the parents.

Cross	Frequency	Phenotypic Character			χ^2	P
		Red Group	Orange Group	White Group		
UH 507 (P) x Manoa Mist (W) (MmOo)* (mmoo)	Observed	11	14	18		
	Expected (1:1:2)	10.75	10.75	21.5	1.12	.70
UH 507 (P) x A 360-88 (R) (MmOo) (MmOo)	Observed	45	15	12		
	Expected (9:3:4)	40.5	13.5	18	2.15	.50
DeWeese (W) x Manoa Mist (W) (mmoo) (mmoo)	Observed			63		
	Expected			63		
Manoa Mist (W) x A220 (W) (mmoo) (mmoo)	Observed			56		
	Expected			56		
Kanda (P) x Manoa Mist (W) (MmOo) (mmoo)	Observed	16	29	46		
	Expected (1:1:2)	22.75	22.75	45.5	3.17	.30

Table 11. (Continued) Segregation of offspring of crosses which have UH 507, A 360-88 or 'Manoa Mist' as one of the parents.

Cross	Frequency	Phenotypic Character			χ^2	P
		Red Group	Orange Group	White Group		
Hirose (P) x Manoa Mist (W) (MmOo) (mmoo)	Observed	42	11	30		
	Expected (1:1:2)	20.75	20.75	41.5	27.79	.001
UH 16 (P) x Manoa Mist (W) (MmOo) (mmoo)	Observed	34	16	31		
	Expected (1:1:2)	20.25	20.25	40.5	11.36	.01
Calypso (P) x Manoa Mist (W) (MmOo) (mmoo)	Observed	5	7	10		
	Expected (1:1:2)	5.5	5.5	11	.2	.95

* Hypothesized genotypes.
Key: P = pink, W = white, R = red.

13 the flavone concentration has no obvious interaction with anthocyanin concentration as has been noted in Antirrhinum (Jorgensen and Geissman, 1955) where the concentration of cyanidin 3-rutinoside varied inversely with the concentration of aurone. This lack of interaction is very clearly illustrated in scattergrams of the flavone concentration on the concentration of pelargonin (Figures 11, 12, and 13).

The data in Tables 12 and 13 suggest a direct relationship between the concentration of cyanidin 3-rhamnosylglucoside and that of pelargonidin 3-rhamnosylglucoside.

Since the scale of measurement used in this analysis is OD (optical density) rather than milligram, and since it has been demonstrated that at pH 3.16 an increase of cyanin from 10^{-4} to 10^{-2} M effects a 300-fold increase in absorbance (Asen et al., 1972), an exponential relationship may be an effect of scale which can be corrected by a logarithmic transformation (Falconer, 1960). Such a transformation of the data was performed and scattergrams of the transformed data plotted (Figures 14, 15 and 16). These scattergrams very clearly demonstrate two groups. The first is one in which there appears to be a direct linear relationship between the cyanin and the pelargonin. The second is one in which there is indication of no relationship between the concentrations of the pigments. These two groups define the two color groups present in anthurium spathes, the red group (pink to dark red) and the orange group (light coral to orange). These discretely different relationships may have some implications for gene action.

The logarithmically transformed absorption data of the progenies analyzed were plotted as histograms and it was found that the cyanin

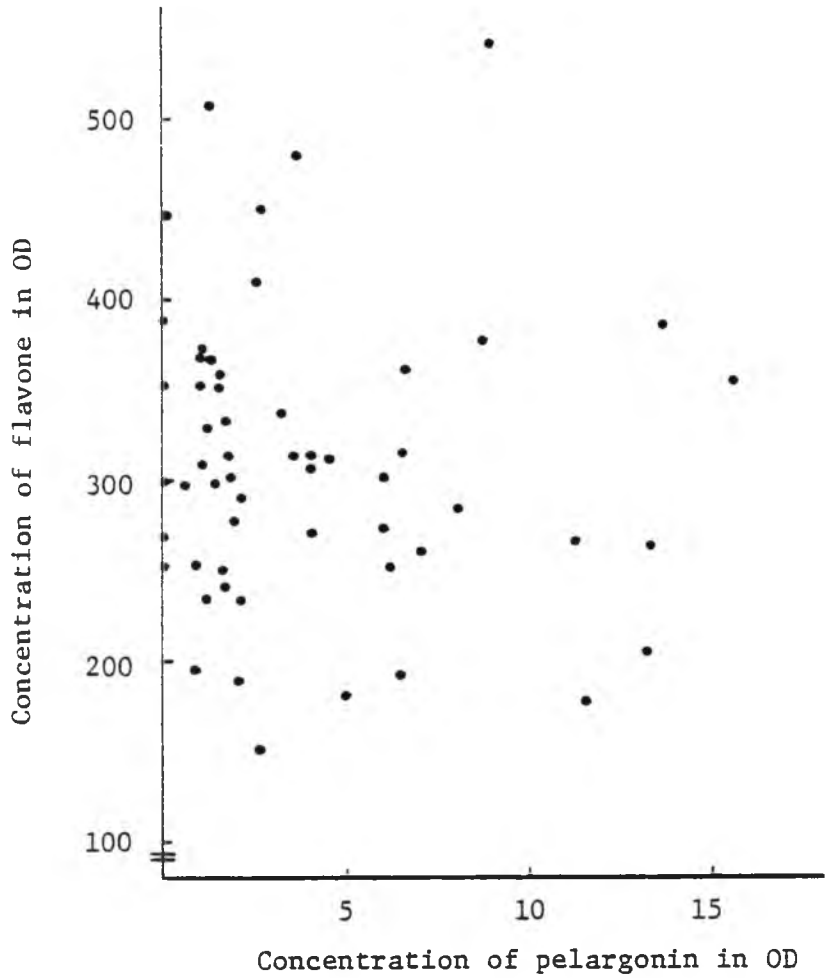


Figure 11. Scattergram of flavone concentration on pelargonin in the progeny of Cross 457.

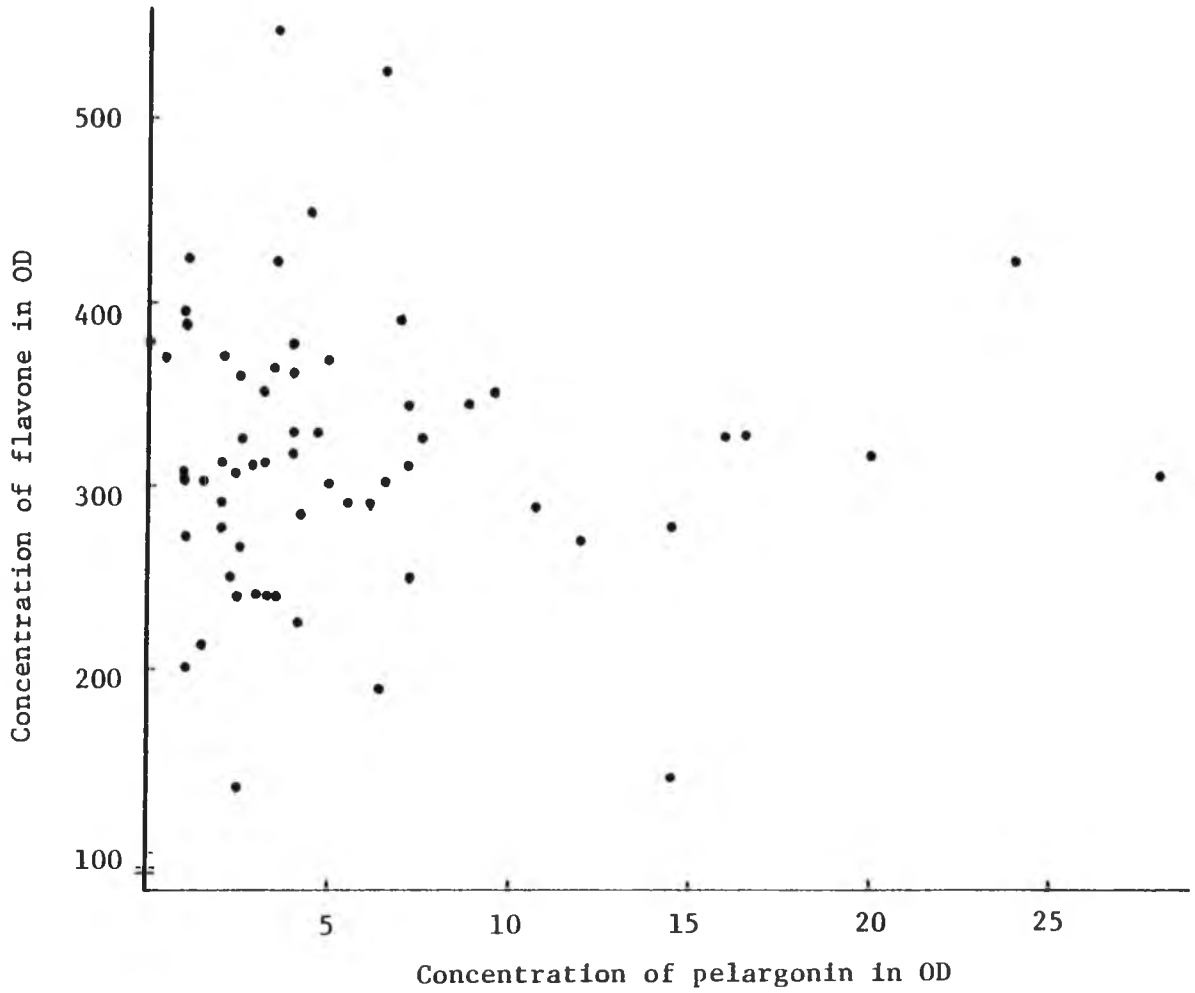


Figure 12. Scattergram of flavone concentration on pelargonin in the progeny of Cross 464.

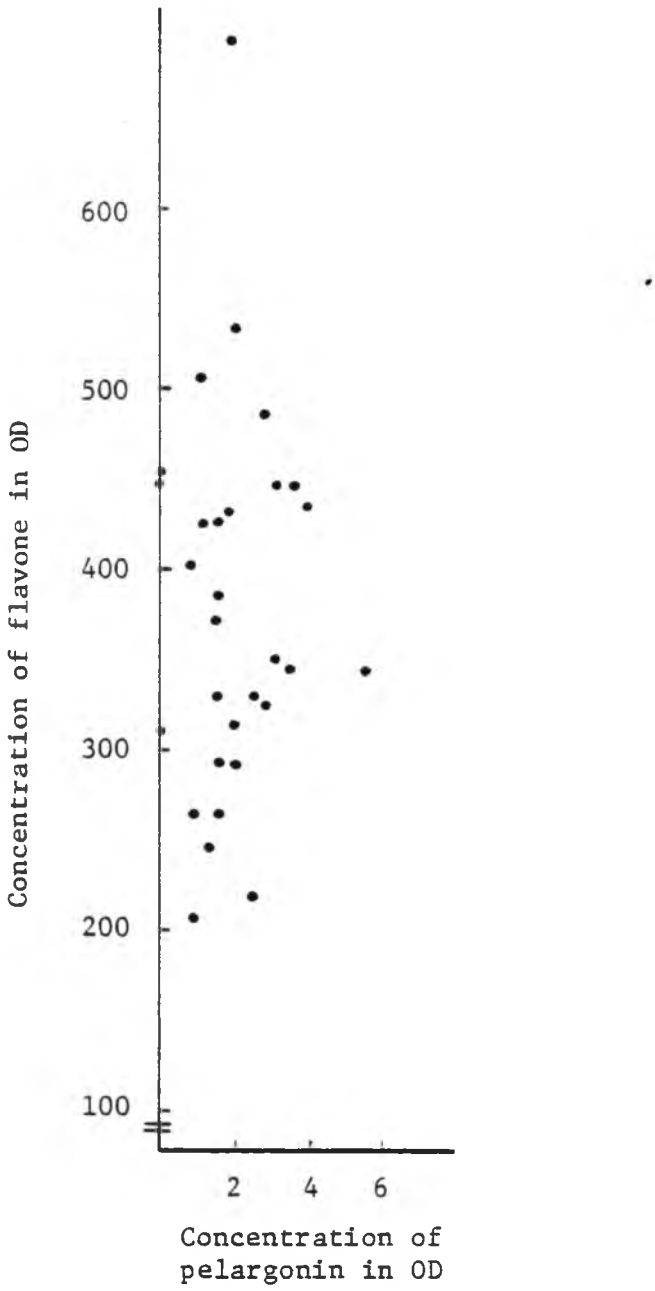


Figure 13. Scattergram of flavone concentration on pelargonin in Cross 435.

Table 12. OD ranges and means of color phenotypes of offspring in crosses 457 and 464.

Cross	Number of Clones	Phenotype	Flavone (nm)		Cyanin (nm)		Pelargonin (nm)	
			Range	Mean	Range	Mean	Range	Mean
457		(Parents)						
		UH 515 (C)	246		0		4	
		Marian Seefurth (P)	243		22		1.2	
	12	R	174-540	322	32-136	70	1.6-13.6	8
	11	LR or DP	150-366	269	14-40	26	0.8-3.6	2
	3	P	252-351	290	3.6-14	8	ca. 0-0.8	0.3
	11	W	276-450	334		0		0
	20	LC	180-504	322	0-0.4	ca. 0	ca. 0-5.3	1
	13	C and DC	201-447	315	0-1.6	0.4	2.4-15.6	7

Table 12. (Continued) OD ranges and means of color phenotypes of offspring in crosses 457 and 464.

Cross	Number of Clones	Phenotype	Flavone (nm)		Cyanin (nm)		Pelargonin (nm)	
			Range	Mean	Range	Mean	Range	Mean
464		(Parents)						
		UH 507 (P)	234		25.6		0.7	
		A 360-63 (O)	252		1.6		15.2	
	3	DR	303-324	311	138-432	262	7.2-28	17
	6	BrR	141-522	333	80-172	149	5.2-24	13
	22	R	186-447	301	22-136	60	2-10.8	4
	6	LR	273-426	332	30-52	38	0.8-6.8	3
	10	W	210-534	357				
	12	LC	128-396	310	0-0.4	0.03	ca. 0-3.2	1
	14	C	201-547	336	0-0.8	0.1	0.8-7.2	5
	2	O and DC	318-324	321	1.6-8	5	16-20	18

Key: DR = dark red, BrR = bright red, R = red, LR = light red, DC = dark coral, C = coral, LC = light coral, O = orange, W = white.

Table 13. OD ranges and means of color phenotypes of offspring in the cross 435.

Cross	Number of Clones	Phenotype	Flavone (nm)		Cyanin (nm)		Pelargonic (nm)	
			Range	Mean	Range	Mean	Range	Mean
435		(Parents)						
		Manoa Mist (W)		330		0		0
		A 360-88 (LR)		342		47		29.6
	6	R	222-486	373	29.4-58	46	1.2-5.6	3
	10	LR	294-534	401	12-32	26	1.2-3.2	2
	3	P	264-411	323	8-18	13	0.8-2.0	1
	29	W	234-648	420		0		0
	13	LC	210-693	362	0-ca. 0	ca. 0	ca. 0-3.6	1
	2	C	294-444	369	0-ca. 0	ca. 0	1.6-3.6	3

Key: R = red, LR = light red, P = pink, W = white, LC = light coral, C = coral.

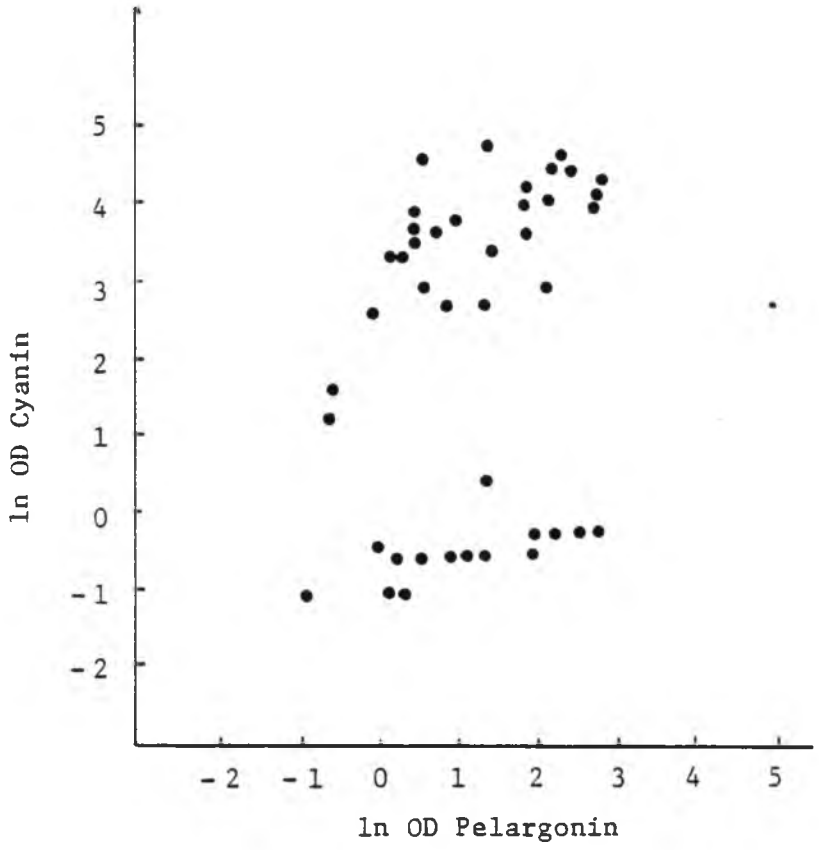


Figure 14. Scattergram of logarithmically transformed data of concentration of cyanin on pelargonin in Cross 457.

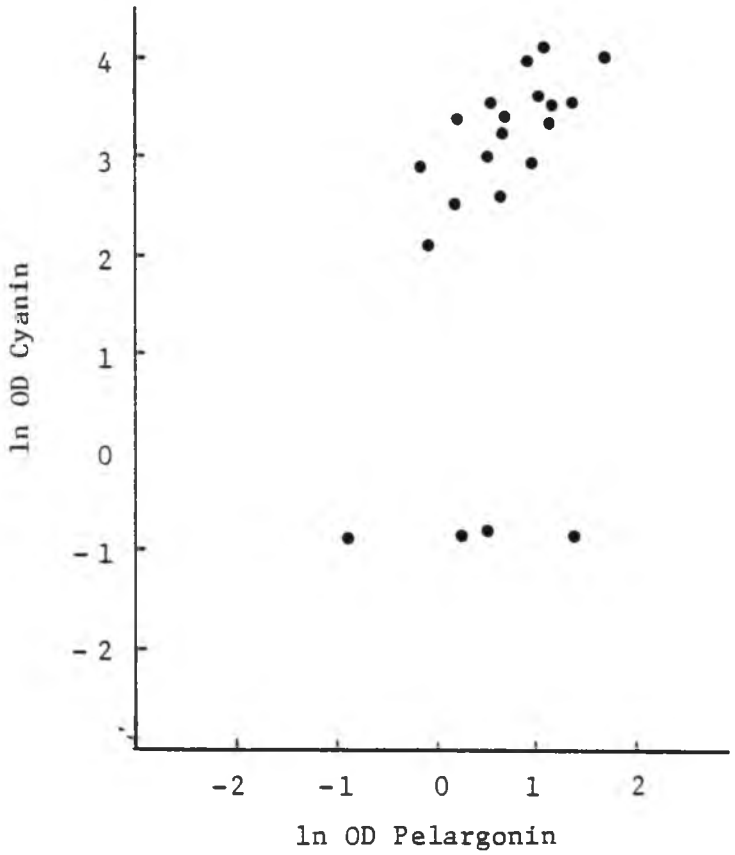


Figure 15. Scattergram of logarithmically transformed data of concentration of cyanin on pelargonin in Cross 435.

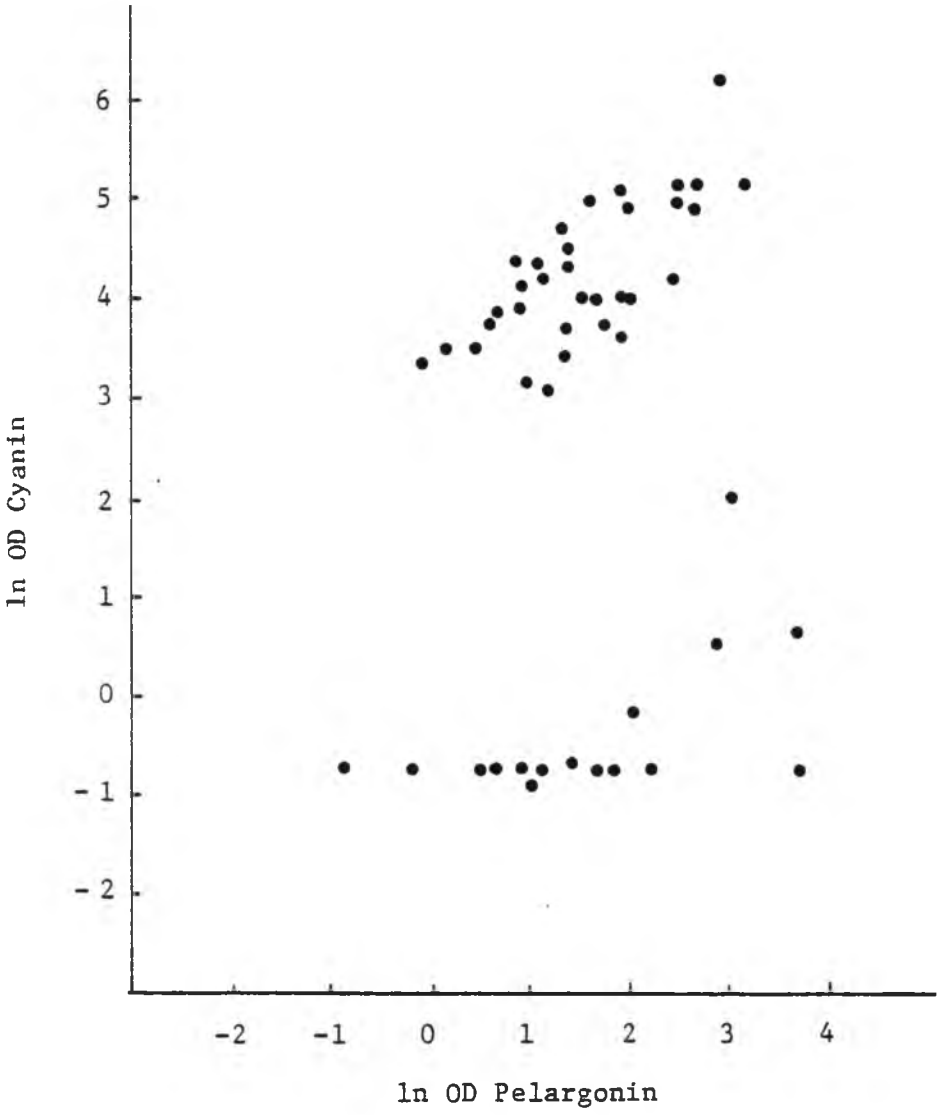


Figure 16. Scattergram of logarithmically transformed data of concentration of cyanin on pelargonin in Cross 464.

pigment was bimodal in all three progenies (Figures 17, 18 and 19). This observation reflects that noted on the scattergrams of these same values in which the higher values of cyanin were directly related to pelargonin concentration but the lower values of the cyanin showed no relationship to the pelargonin concentration.

It was noted in section 4.3 (Table 10) that there was poor fit of the biochemically scored progeny ratios to the expected ratios based on the hypothesized parent genotypes. On the other hand there was good fit with the visually scored progeny ratios. In other words, there is relationship between genotype, visually scored phenotype, and the cyanin pigment group which has a higher concentration. No similar relationship is evident with the cyanin pigment group which has the very low concentrations.

These findings suggest that the minor amounts of the cyanin pigment are not under the control of the dominant M allele. This seems to explain the fit obtained with the visually rated progeny groups rather than the biochemically scored segregations. The low levels of cyanin seem to have a random characteristic, possibly related to multiple environmental and physiologic influences.

In maize the dominant allele Pr controls 3'-hydroxylation in anthocyanins but some of the 3'-hydroxylated pigment appears in the allelic forms indicating that the allele is a hypomorph (Harborne and Gavazzi, 1969). This is not a common situation in the genetics of higher plants, but it has been found in Solanum in relation to flavonol production. It is suggested that the m allele in A. andraeanum is also a hypomorph which implies that it is a suppressor of cyanidin synthesis

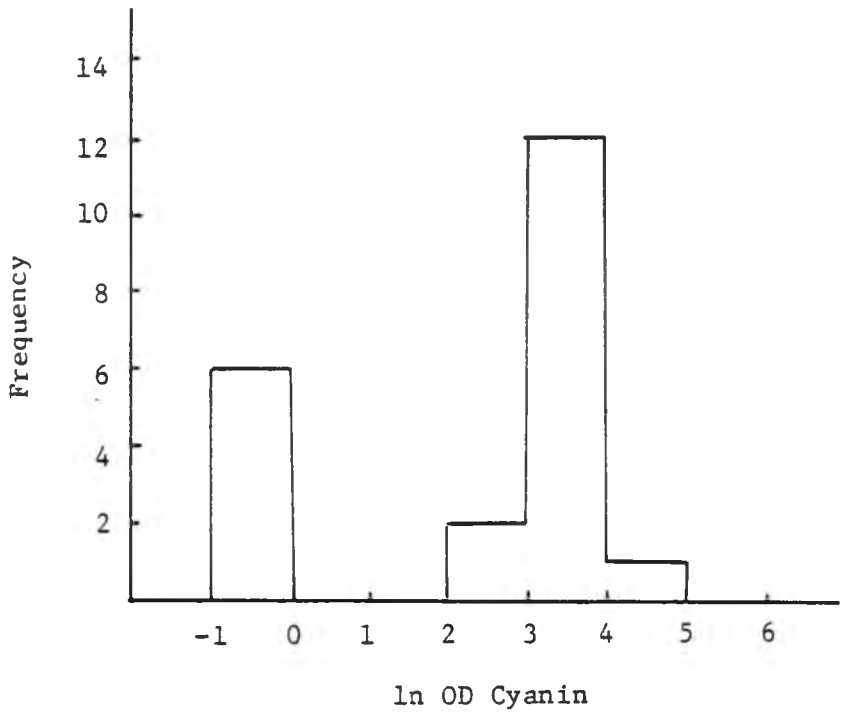


Figure 17. Histogram of logarithmically transformed data of the concentrations of cyanin in Cross 435.

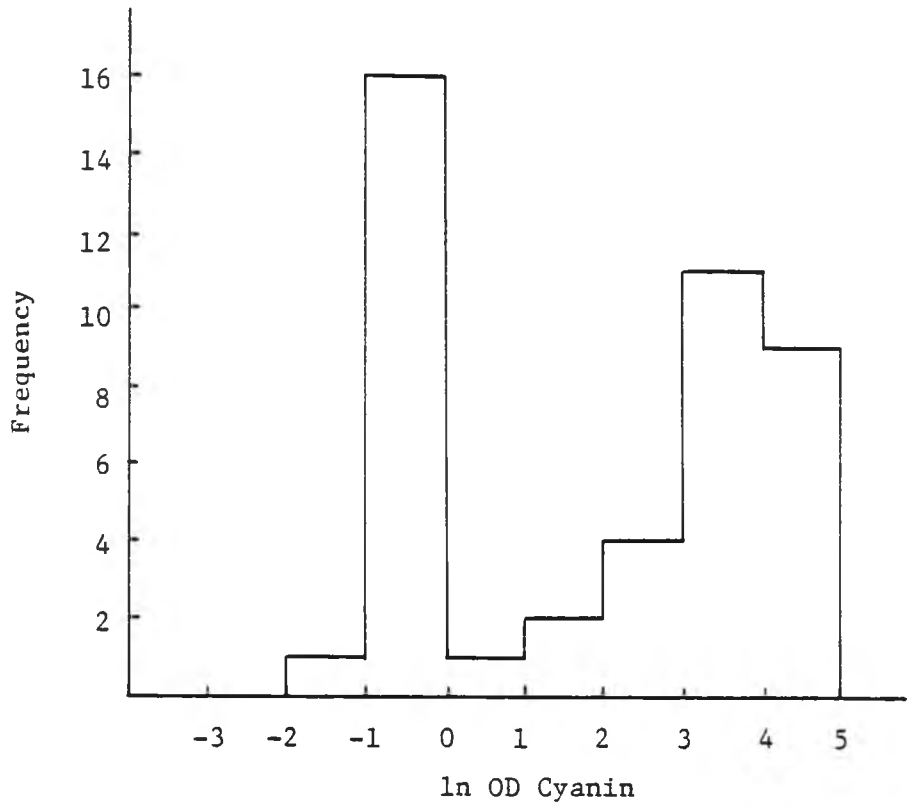


Figure 18. Histogram of logarithmically transformed data of the concentrations of cyanin in Cross 457.

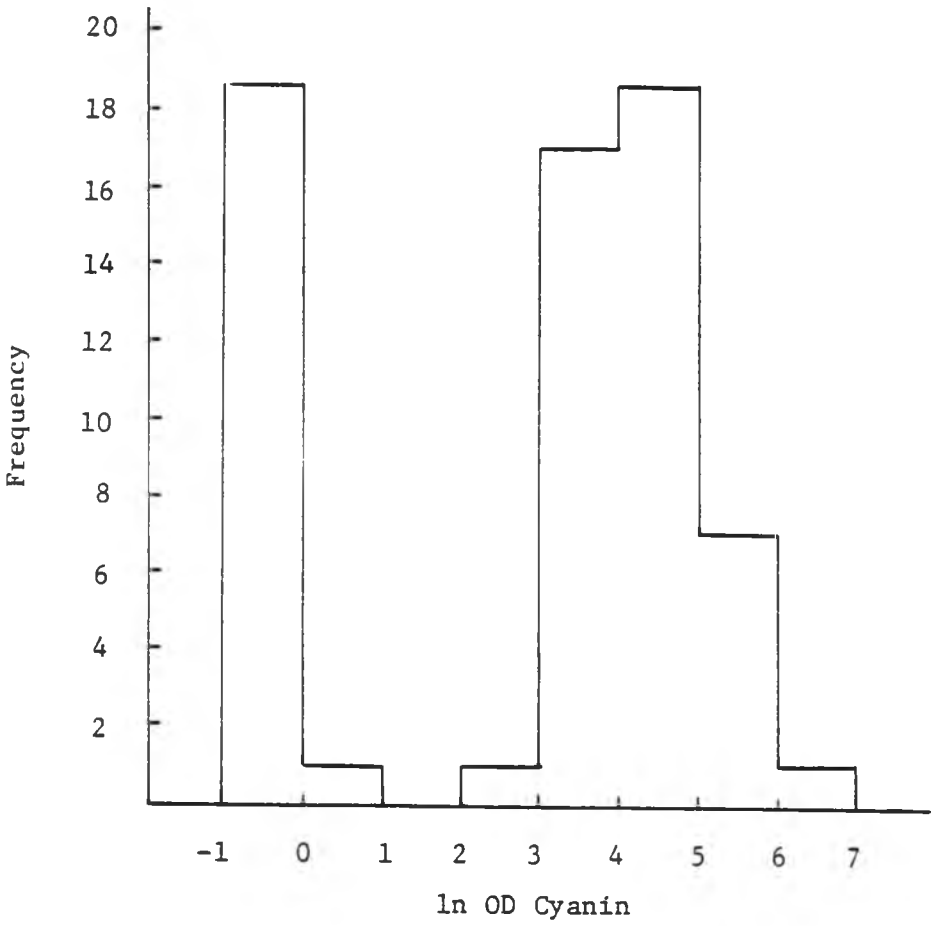


Figure 19. Histogram of logarithmically transformed data of the concentrations of cyanin in Cross 464.

rather than a null allele. It is also possible that the infrequency of such situations in higher plants may be partly related to techniques of quantitation available to the investigator. In this study use of TLC and a solvent system which gave excellent resolution allowed the detection of very small quantities of anthocyanins.

Gene dosage effect involving incomplete dominance is postulated. In such a system the dominant homozygote would possess the most intense concentration of pigment, the heterozygote an intermediate concentration and the recessive homozygote, essentially none.

Jorgensen and Geissman (1955) demonstrated in Antirrhinum that with increasing recessive factors, there is a decrease in anthocyanin production. The factors included one which controlled aurone concentration. In that particular situation the aurone production was competitive with anthocyanin production so that the anthocyanin production was essentially influenced because the recessive gene stimulated aurone production whereas the dominant anthocyanin genes controlled anthocyanin synthesis. Nevertheless it has been a general observation that there is enhanced anthocyanin production with increased dominant pigment genes (Harborne, 1967). Lawrence and Sturgess (1957) found that penetrance of the genes controlling anthocyanin production increased with the increase in the number of dominant genes so that or < oR < OR in which O controlled 3'-, 5'-hydroxylation and R controlled 3'-hydroxylation of anthocyanins. In that system the incremental effects of R and O were equal. It is postulated that a similar condition is operative in the anthurium system but that the incremental effect of M is greater than O so that mmoo = Mmoo = MMoo < mmOo < mmOO < MmOo <

MmOO < MMOo < MMOO.

In Crosses 457 and 464 one of the expected genotypes in the progenies has three dominant alleles (MmOO) whereas in Cross 435 this is not expected. In cross 435 quantitative analysis showed that the highest concentrations fell in the 3-4 category (Figure 17). In Cross 457 the highest concentrations fell in the 4-5 category (Figure 18) and in Cross 464 a number fell in the 4-6 categories (Figure 19).

In Crosses 457 and 464 one of the expected genotypes for the orange group phenotype has two dominant alleles (mmOO). In Cross 435 the only genotype corresponding to the orange group is mmOo having only one dominant allele. The preponderance of plants in the orange group of Cross 435 lies in the Light Coral classification (Table 13). In Crosses 464 and 457 there are a large number in the Coral or Dark Coral classification (Table 12). It is likely that there are a number of other factors involved in the determination of pigment concentration and/or visual effect. Consideration of such factors is beyond the scope of this work.

The proposed scheme of inheritance of spathe color takes into account qualitative and quantitative variations in spathe pigments. The scheme consists of a system of monogenic control of each anthocyanin, an incomplete dominance form of interallelic interaction involving dosage effects on the concentration of each pigment, and recessive epistasis.

The flavone was present in substantial quantity in every plant analyzed chemically, so that segregation appears to be only in quantitative terms. There has been too little data on the behavior of flavone to even begin to speculate on inheritance mechanisms. Therefore

no attempt was made to do so. The role of the flavone in spathe color variation in anthurium has not been demonstrated. Its role probably does not go beyond stabilization of the anthocyanins as suggested by Asen *et al*. (1972).

APPENDIXES

APPENDIX I

Estimation of the Reliability and Variability of Quantitative Measurements of Pigments in the Spathes of Anthurium

A measure of reliability of the method used in this study for quantitative measurements was estimated by isolating the pigments from the same extract a number of times and determining the OD of each pigment. The results are presented in Table 14 and demonstrate high reliability.

A measure of the variability of the quantitative measurement of anthocyanins within a clone was estimated by sampling spathes from different plants of the same clone on the same day. Results are presented in Table 15. Expected variability exists but within tolerable limits.

A measure of the seasonal variability which occurs in the quantitative measurement of anthocyanins in one clone was estimated by taking measurements of spathe extracts from one clone at different times of the year. This data is presented in Table 16.

Table 14. Quantitative measurements taken of pigments isolated from a single extract but different isolation runs.

Run	OD/Cyanin	OD/Pelargonin
1	0.9	0.30
2	0.94	0.38
3	0.98	0.35
4	0.96	0.32
5	0.96	0.32
Mean ± SE	0.95 ± .015	0.34 ± .014

Table 15. Quantitative measurements taken of pigments from different plants of the same clone but on the same day.

	Sample	OD/Cyanin	OD/Pelargonin
Kaumana	A	1.12	0.39
	B	1.02	0.34
	C	0.94	0.31
	D	0.92	0.32
	Mean ± SE	0.95 ± .045	0.34 ± .02
	CV	9.0%	10.5%

Table 16. Quantitative measurements taken of pigments from different plants of the same clone at different times during the year.

Sample	OD/Cyanin	OD/Pelargonin
Kaumana - March	0.98	0.35
Kaumana - June	1.17	0.3
Kaumana - August	0.9	0.22
Kaumana - January	1.0	0.26
Mean ± SE	1.01 ± .057	0.28 ± .048
CV	11%	20%

APPENDIX II

Spectra of the Compounds Isolated by Two-Dimensional Chromatography of the Methanolic Extracts of anthurium spathes

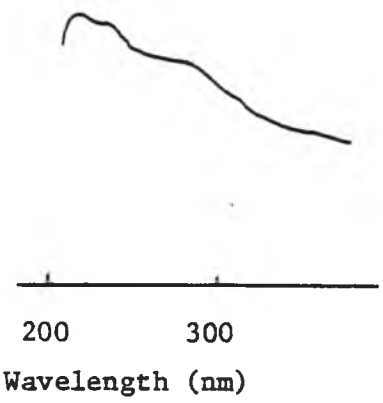


Figure 20. Spectrum of Spot No. 1 - 214, 236 nm.

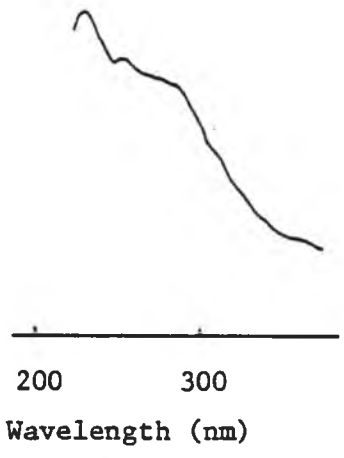


Figure 21. Spectrum of Spot No. 2 - 230, 260 nm.

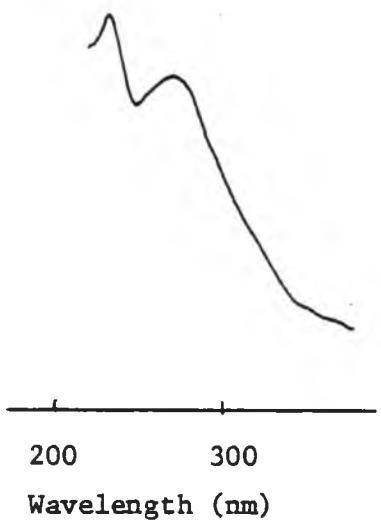


Figure 22. Spectrum of Spot No. 3 - 230, 270 nm.

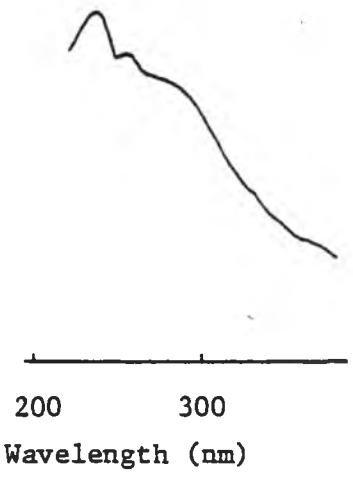


Figure 23. Spectrum of Spot No. 4 - 230, 260 nm.

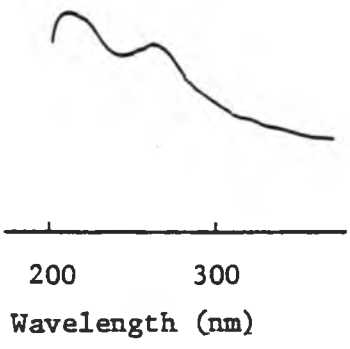


Figure 24. Spectrum of Spot No. 5 - 218, 270 nm.

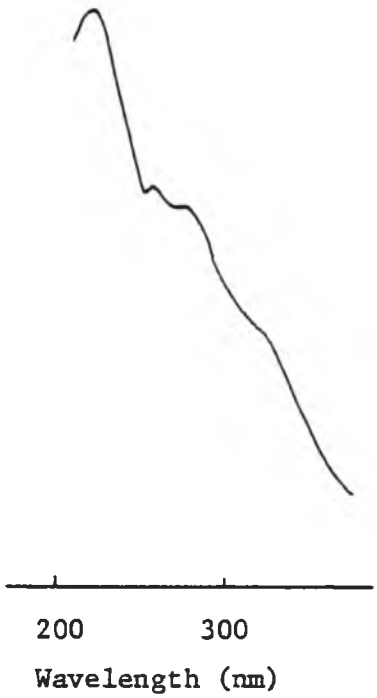


Figure 25. Spectrum of Spot No. 6 - 216, 278 nm.

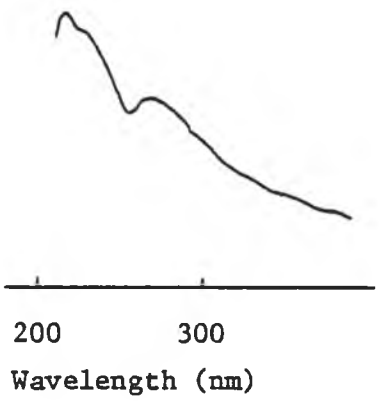


Figure 26. Spectrum of Spot No. 9 - 210, 270 nm.

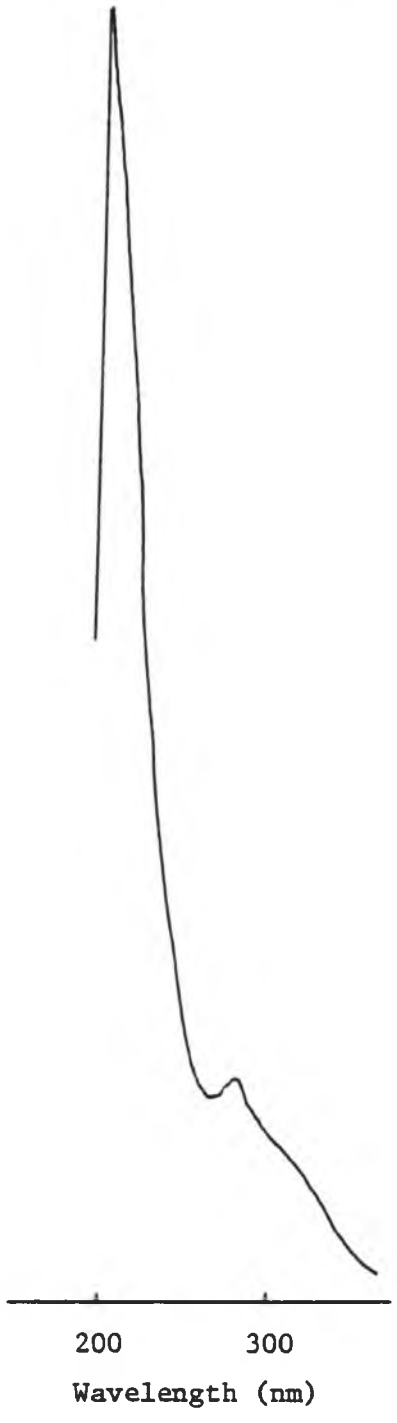


Figure 27. Spectrum of Spot No. 10 - 210, 282 nm.

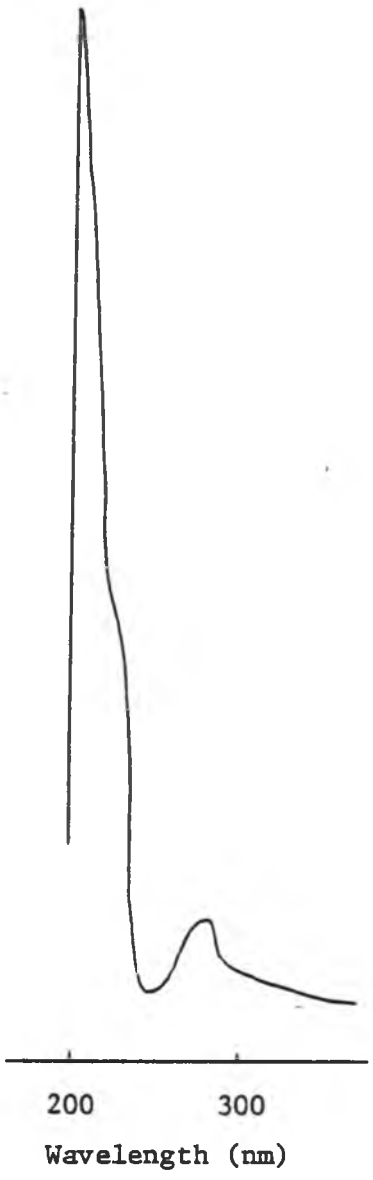


Figure 28. Spectrum of Spot No. 11 - 208, 284 nm.

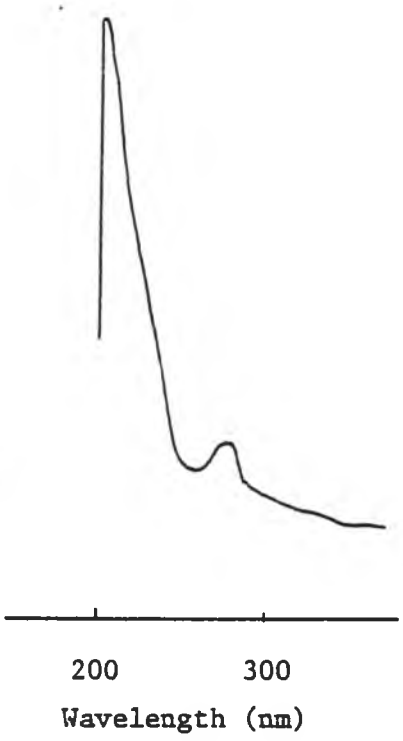


Figure 29. Spectrum of Spot No. 12 - 208, 280 nm.

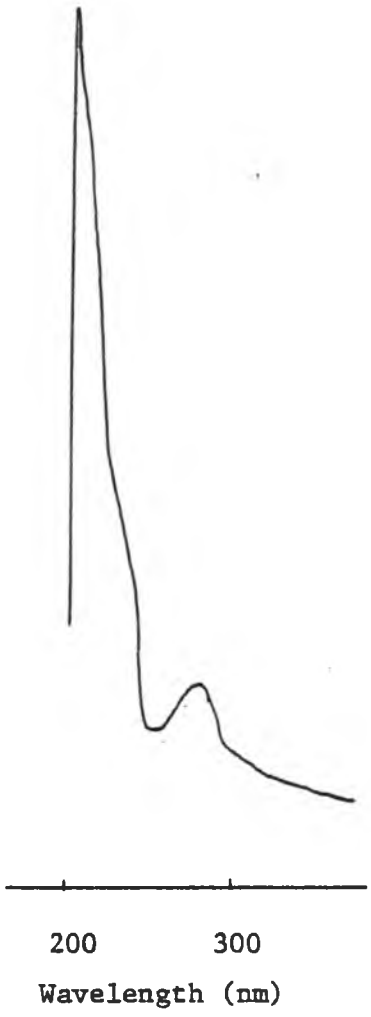


Figure 30. Spectrum of Spot. No. 13 - 210, 284 nm.

Figure 31. Spectrum of Spot No. 14 - 210, 270 nm.

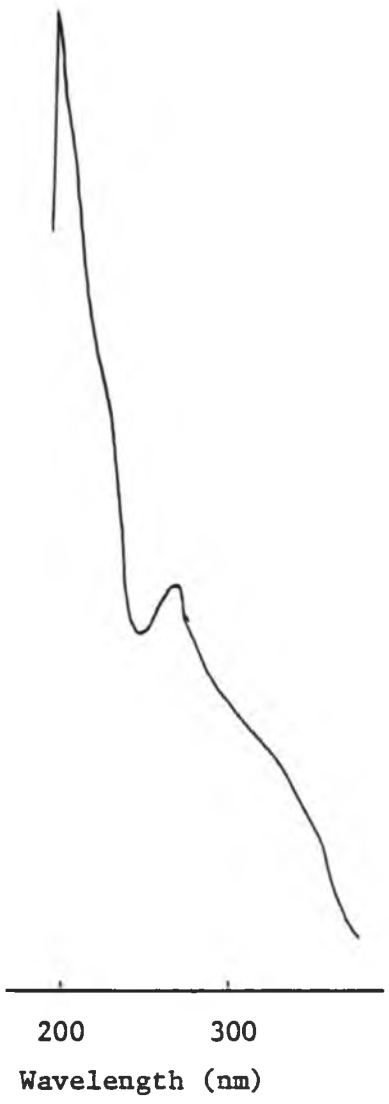


Figure 32. Spectrum of Spot No. 15 - 210, 270 nm.

Figure 33. Spectrum of Spot. No. 16 - 210 nm.

APPENDIX III

Concentrations of Anthocyanins and Acacetin 7-glycoside Expressed as OD per gram Fresh Weight in Clones of *Anthurium andraeanum* in the Breeding Stock at the University of Hawaii

Color	Anthurium Plant	Flavone	Cyanin	Pelargonin
Dark Red	A 147 Tsutsui Red	324	432	16.4
	A 26 Kaumana	396	392	14.0
	UH 448	216	254	20.4
	UH 377	309	324	21.0
	UH 437	299	224	26.4
	UH 538	299	304	24.8
	UH 27	252	340	10.4
	A 98 Kozohara	342	264	7.2
	UH 438	402	232	2.9
	A 110 Kansako No. 1	420	216	5.6
	A 159 Murayama Red	306	352	27.0
	UH 126	252	504	12.4
	A 138 Nakazawa Red	276	280	12.0
Bright Red	UH 493 (Obake)	276	180	4.8
	UH 112	210	142	50.0
	UH 68	270	156	34.0
	A 106 Hirose Red	297	184	24.0
	UH 163	294	168	27.0
	A 65 Hayashi Red	360	149	8.8
	A 72	342	160	16.4

APPENDIX III (Continued)

Concentrations of anthocyanins and flavone in A. andraeanum.

Color	Anthurium Plant	Flavone	Cyanin	Pelargonin
Bright Red (continued)	A 154	180	136	10.4
	A 155	222	196	10.4
	A 99 Fukano Red	204	134	29.6
Red	UH 443	216	132	7.6
	A 71 Asato Red	147	126	9.6
	UH 475	261	154	12.0
	A 178 Mickey Mouse	192	66	5.6
	UH 522	240	58	1.6
	434 (Interspecific Hybrid)	150	80	4.8
Light Red	A 75	426	23	4.5
	A 72 Ozaki	153	64	12.4
	A 111 Kansako No. 2	162	88	14.4
Dark Pink	UH 186	345	60	10.4
	A 360-88	342	30	2.0
	A 132 Abe Pink	303	48	4.0
	UH 383	312	18	0.5
Pink	UH 33 Marian Seefurth	243	22	1.2
	UH 507	257	26	0.7
	A 163 Kanda Pink	108	18	1.2
	A 356 Tatsuta Pink Obake	252	34	3.6
White	Manoa Mist	330	0	0

APPENDIX III (Continued)

Concentrations of anthocyanins and flavone in A. andraeanum.

Color	Anthurium Plant	Flavone	Cyanin	Pelargonin
Light Coral	A 146 Fujii Light Pink	360	0	0.8
Dark Coral or Light Orange	Anuenue	204	1.6	13.6
	UH 515	246	0	4.0
	A 153	198	ca. 0	7.2
Orange	A 157 Okamoto	210	ca. 0	40.0
	A 93 Nitta	372	0	64.0
	A 360-63	252	1.6	15.2
	UH 139	279	4.8	66.4

APPENDIX IV

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Red			
	174	88	11.6
	300	44	1.6
	414	44	2.4
	540	88	8.8
	480	32	3.6
	264	76	13.2
	381	68	13.6
	282	65.6	8.0
	300	136	6.3
	192	60	6.4
	267	42	6.0
	264	96	11.2
Dark Pink or Light Red			
	318	16	3.6
	366	28	1.36
	330	40	1.6
	249	20	1.6
	186	20	0.8
	150	16	2.4
	324	26	1.2

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Dark Pink or Light Red (continued)			
	252	14	0.8
	240	40	1.6
	234	38	2.0
	312	32	1.6
Pink			
	351	14	0.8
	252	6	ca. 0
	267	3.6	ca. 0
Coral and Dark Coral			
	354	0.8	15.6
	261	0.8	7.2
	360	0	6.4
	303	ca. 0	4.0
	270	1.6	4.0
	312	0	6.4
	201	0.8	13.2
	447	ca. 0	2.4
	312	ca. 0	6.4
	378	0.8	8.8

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Coral and Dark Coral (continued)			
	312	ca. 0	4.0
	252	ca. 0	6.4
	336	ca. 0	3.2
Light Coral			
	372	0	1.1
	309	0	4.8
	351	ca. o	1.4
	297	ca. 0	ca. 0
	504	0	1.2
	279	0	1.8
	444	0	ca. 0
	384	0	ca. 0
	384	0	ca. 0
	306	0	0.8
	180		5.33
	288	0.4	2.0
	234	ca. 0	1.2
	297	0	0.6
	186	0	2.0

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Light Coral (continued)			
	369	ca. 0	1.0
	297	ca. 0	1.4
	252	0	ca. 0
	357	ca. 0	1.6
	351	0	0.1
White			
	315	0	0
	300	0	0
	282	0	0
	450	0	0
	396	0	0
	414	0	0
	312	0	0
	300	0	0
	279	0	0
	345	0	0
	276	0	0
Dark Red			
	306	138	7.2
	303	216	28.0

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Dark Red (continued)			
	324	432	16.4
Bright Red			
	420	172	24.0
	279	166	14.4
	141	80	14.4
	366	144	5.2
	522	162	6.6
	270	168	12.0
Red			
	186	36	6.4
	303	52	5.2
	240	32	3.6
	312	54	2.4
	369	46	2.0
	312	72	3.2
	282	76	4.2
	288	50	2.0
	240	22	3.2
	252	52	3.2
	447	52.8	4.4

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Red (continued)			
	354	66	9.6
	264	68	2.4
	288	46	2.0
	363	42	3.6
	360	84	2.4
	240	64	3.2
	330	86	4.0
	288	136	10.8
	252	56	7.2
	420	96	3.6
	240	24	2.4
Light Red			
	288	40	5.6
	273	30	0.8
	387	52	6.8
	426	32	1.2
	303	32	1.6
	312	40	3.0

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Coral			
	318	0	4.0
	378	ca. 0	4.0
	225	0	4.2
	324	0	7.6
	342	0.8	7.2
	333	ca. 0	4.8
	360	ca. 0	4.0
	327	ca. 0	2.4
	300	0	6.4
	288	ca. 0	6.2
	342	ca. 0	8.8
	201	ca. 0	0.8
	547	ca. 0	3.52
	420	ca. 0	3.6
Light Coral			
	279	ca. 0	2.0
	294	ca. 0	2.0
	378	0	ca. 0
	354	ca. 0	3.2
	309	0	0.8

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Light Coral (continued)			
	210	ca. 0	1.6
	303	0	0.2
	396	0	0.2
	387	0	0.8
	128	0.4	2.6
	372	ca. 0	0.4
	309	ca. 0	2.4
Orange and Dark Coral			
	318	8	20.0
	324	1.6	16.0
Red			
	486	58	2.8
	324	42	2.8
	429	29	1.2
	342	54	5.6
	222	52	2.4
	435	38	4.0
Light Red			
	372	20	1.6
	330	20	2.4

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Light Red (continued)			
	504	12	1.2
	444	30	3.2
	426	32	1.6
	330	32	1.6
	430	25	1.8
	348	32	3.2
	294	30	2.0
	534	24	2.0
Pink			
	264	18	0.8
	294	14	2.0
	411	8	0.8
Coral			
	444	ca. 0	3.6
	294	0	1.6
Light Coral			
	246	0	1.4
	210	0	0.8
	504	0	1.07

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
Light Coral (continued)			
	387	ca. 0	1.6
	264	0	0.3
	264	ca. 0	1.6
	693	0	1.8
	308	0	0.3
	456	ca. 0	ca. 0
	342	0	3.6
	315	0	2.0
	450	0	ca. 0
	264	0	1.4
White			
	384	0	0
	546	0	0
	390	0	0
	234	0	0
	333	0	0
	528	0	0
	516	0	0
	648	0	0
	551	0	0

APPENDIX IV (Continued)

Concentrations of Anthocyanins and Flavone
Expressed as OD per gram Fresh Weight in Cross 457.

Color	Flavone	Cyanin	Pelargonin
White (continued)			
	378	0	0
	378	0	0
	369	0	0
	600	0	0
	462	0	0
	552	0	0
	390	0	0
	288	0	0
	429	0	0
	378	0	0
	282	0	0
	432	0	0
	531	0	0
	282	0	0
	330	0	0
	384	0	0
	429	0	0
	414	0	0
	390	0	0
	378	0	0

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